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ECBC-TR-326

TOXICITY ASSESSMENTS OF ANTIMONY, BARIUM, BERYLLIUM, AND MANGANESE FOR DEVELOPMENT OF ECOLOGICAL SOIL SCREENING LEVELS (ECO-SSL) USING FOLSOMIA REPRODUCTION BENCHMARK VALUES

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November 2002

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Aberdeen Proving Ground, MD 21010-5424

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*
XX-11-2002

2. REPORT TYPE
Final

3. DATES COVERED *(From - To)*
Feb 2000 – Sep 2002

4. TITLE AND SUBTITLE
Toxicity Assessments of Antimony, Barium, Beryllium, and Manganese for Development of Ecological Soil Screening Levels (Eco-SSL) Using Folsomia Reproduction Benchmark Values

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
9KNM22

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)
DIR, ECBC, ATTN: AMSRD-ECB-RT-TE, APG, MD 21010-5424
CO, NAVFAC, EFANE, 10 Industrial Highway, MS #82, Lester, PA 19113-2090

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-326

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
CO, NAVFAC, EFANE, 10 Industrial Highway, MS #82, Lester, PA 19113-2090

10. SPONSOR/MONITOR'S ACRONYM(S)
NAVFAC, EFANE

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The U.S. Environmental Protection Agency is developing Ecological Soil Screening Level (Eco-SSL) benchmarks for ecological risk assessment of contaminants at Superfund sites. Benchmarks for invertebrates were developed from existing literature. Insufficient information for barium (Ba), beryllium (Be), manganese (Mn), and antimony (Sb) to generate Eco-SSLs necessitated standardized toxicity testing to fill the data gaps. We used the Folsomia [*Folsomia candida* (*F. candida*)] Reproduction Test in this study. This test was selected on the basis of its ability to measure chemical toxicity to ecologically relevant test species during chronic assay, and its inclusion of at least one reproductive component among the measurement endpoints. Tests were conducted in Sassafras sandy loam soil, which supports relatively high bioavailability of metals. Weathering/aging procedures for spiked treatment soil were incorporated into the study to better reflect the "real world" exposure conditions. Definitive toxicity tests conducted with aged/weathered soils amended with test chemicals showed that chemical toxicity order based on Lowest Observed Effect Concentration (LOEC) values for juvenile production in tests with *F. candida* was Be > Sb > Ba > Mn with EC_{20} values of 28, 81, 165, and 1209 mg kg^{-1}, respectively. These tests were conducted under conditions preferred for Eco-SSL derivation, using a soil that supports relatively high bioavailability of Ba, Be, Mn, and Sb.

15. SUBJECT TERMS

Barium	Antimony	Bioavailability	Ecological Soil Screening Level
Beryllium	Toxicity assessment	Natural soil	
Manganese	Weathering/aging	*Folsomia candida*	

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON / 19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UL	81	Sandra J. Johnson / (410) 436-2914

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PREFACE

The work described in this report was authorized under Sales Order No. 9KNM22. The work was started in February 2000 and completed in September 2002.

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Acknowledgments

This project was completed in cooperation with and from funding provided by the Engineering Field Activity Northeast (EFANE), Naval Facilities Engineering Command (Lester, PA).

The authors thank Stephen J. Ells for support and assistance, and acknowledge the Ecological Soil Screening Level National Program, administered under the auspices of the Office of Solid Waste and Emergency Response (OSWER), U.S. Environmental Protection Agency (USEPA), Washington, DC.

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TOXICITY ASSESSMENTS OF ANTIMONY, BARIUM, BERYLLIUM, AND MANGANESE FOR DEVELOPMENT OF ECOLOGICAL SOIL SCREENING LEVELS (ECO-SSL) USING FOLSOMIA REPRODUCTION BENCHMARK VALUES

1. INTRODUCTION

The U.S. Environmental Protection Agency (USEPA) is developing Ecological Soil Screening Levels (Eco-SSLs) for ecological risk assessment of contaminants at Superfund sites. Eco-SSLs are soil concentrations of chemicals which, when not exceeded, will theoretically protect terrestrial ecosystems from unacceptable harmful effects. They are derived using data generated from laboratory toxicity tests with different test organisms, which represent the vast array of ecological receptors. Whenever sufficient quantity and quality of information existed, Eco-SSLs for soil invertebrates were developed from studies reported in literature. However, insufficient information to generate Eco-SSLs for barium (Ba), beryllium (Be), Manganese, (Mn), and antimony (Sb) necessitated standardized toxicity testing to fill the data gaps.

This study was designed to produce benchmark data for the development of an Eco-SSL for Ba, Be, Mn and Sb for soil invertebrates, and meet specific criteria (USEPA, 2000), including: (1) tests were conducted in soil having physicochemical characteristics that support relatively high bioavailability of metals; (2) experimental designs for laboratory studies were documented and appropriate; (3) both nominal and analytically determined concentrations of chemicals of interest were reported; (4) tests included both negative and positive controls; (5) chronic or life cycle tests were used; (6) appropriate chemical dosing procedures were reported; (7) concentration-response relationships were reported; (8) statistical tests used to calculate the benchmark and level of significance were described; and (9) the origin of test species were specified and appropriate.

Several soil invertebrate toxicity tests, for which standardized protocols have been developed, can effectively be used to assess the toxicity and to derive protective benchmark values for metals (Stephenson *et al.* 2000; Løkke and Van Gestel, 1998). We used the Folsomia Reproduction Test in these studies. This test was selected on the bases of its ability to measure chemical toxicity to ecologically relevant test species during chronic assays, and its inclusion of at least one reproductive component among the measurement endpoints.

Special consideration in assessing chemical toxicity for Eco-SSL development was given to the effects of weathering/aging of soil contaminants on the exposure of relevant ecological receptors, as commonly occurs at Superfund sites. During chemical weathering/aging in soil, reduction in the exposure to the chemical may occur due to volatilization, microbial degradation and immobilization, or other fate processes (e.g., photodecomposition, hydrolysis, and hysteresis, etc.). This can result in a dramatic reduction in the amount of chemical that is bioavailable, compared to tests conducted with freshly-amended chemicals or those tested following a short equilibration period (e.g., 24 h). Standardized methods for weathering/aging of chemicals in soil are not available. We used the approach developed to simulate at least partially, the aging and weathering process that included exposing soils amended with chemicals to periodic alternating wetting and air-drying cycles for 3 weeks, in a green house.

2. MATERIAL AND METHODS

2.1 Test Soil.

A natural soil, Sassafras sandy loam [Fine-loamy, siliceous, mesic Typic Hapludult] (SSL) was used in this study to assess the toxicity of test chemicals to *F. candida*. This soil was selected for developing ecotoxicological values protective of soil biota because it has physical and chemical characteristics supporting relatively high bioavailability of the test chemicals (low pH, organic matter and clay contents). The SSL soil was collected from an open grassland field on the property of the U.S. Army Aberdeen Proving Ground (APG; Edgewood, MD). Vegetation and the organic matter horizon were removed to just below the root zone and the top six inches of the A horizon were then collected. The soil was sieved through a $5mm^2$ mesh screen, air-dried for at least 72 h and mixed periodically to ensure uniform drying, passed through a 2-mm sieve, and stored at room temperature before use in testing. Soil was analyzed for physical and chemical characteristics. Results showed this soil was 71% sand, 18% silt, 11% clay, a CEC of 4.27 cmol kg^{-1}, pH of 5.0 and an organic matter content of 1.2% (analyzed by the Cooperative Extension Service, University of Maryland Soil Testing Laboratory, College Park, MD).

2.2 Test Chemicals.

The goal of these studies was to determine the toxicity of Ba, Be, Mn, and Sb to *F. candida*. Assessments were done using sulfate salts, including $BaSO_4$ (CAS #7727-43-7, 97%; stock #13989; lot #I10J20, Alfa Aesar), $BeSO_4*4H_2O$ (CAS #7787-56-6, 99.99%; stock #16104; lot #H09J07, Alfa Aesar), $MnSO_4*H_2O$ (CAS #10034-96-5, ACS, 98.0-101.0%, stock #33341; lot #I18I29, Alfa Aesar), and $Sb_2(SO_4)_3$ (CAS #7446-32-4, 97%, stock #33492; lot #L21I28, Alfa Aesar). Additional tests were done for Ba and Sb to determine how carrier salts and their relative solubilities affect the toxicity to *F. candida*. For Ba, these compounds including BaO (CAS #1304-28-5, 97%, lot #12101BI, Aldrich Chemical Company), $Ba(NO_3)_2$ (CAS #10022-31-8, ACS, lot #000420, Fisher Scientific Co.), and $Ba(C_2H_3O_2)_2$ (CAS #543-80-6, ACS, lot #995963, Fisher Scientific Co.). For Sb, we used antimony D-tartrate $Sb_2(C_4H_4O_6)_3*6H_2O$ (CAS # 126506-93-2, lot #111004-2, Pfaltz & Bauer). The positive control used in these studies was Prentox® carbamate 1.5 EC (Prentiss Drug & Chemical Co., Inc., Floral Park, NY). The main carrier salt control was sulfate as $CaSO_4*2H_2O$ (CAS #10101-41-4, ACS, Reagent grade 100%, lot #C07704, J.T. Baker). Purified water (ASTM type I; American Society of Testing and Materials, http://www.astm.org) obtained using Milli-RO® 10 Plus followed by Milli-Q® PF Plus systems (Millipore®, Bedford, MA) was used throughout the studies.

2.3 Soil Amendment Procedures.

Treatment concentrations for toxicity tests with all sulfate salts and barium oxide were prepared by adding test chemicals to SSL soil in appropriate proportions to achieve nominal target concentrations. Soil was mixed for 3 h on a three dimensional rotary mixer. After mixing, soil was hydrated with purified water to 88% of the soil water holding capacity (WHC; 18% water, on the basis of dry soil mass) for toxicity testing, both range-finding and definitive studies. Soils were wetted up to 60% of the WHC during the weathering/aging procedure. Soil prepared for testing was allowed to equilibrate for 24 h before introducing the springtails, *Folsomia candida*. The exception was soil amended with barium acetate, which was incubated for 5 days before exposing springtails to allow acetate degradation by soil microbes. Treatment concentrations of $Ba(C_2H_3O_2)_2$, $Ba(NO_3)_2$ and $Sb_2(C_4H_4O_6)_3$ were prepared by dissolving appropriate amounts of each chemical in purified water, then hydrating pre-weighed amounts of SSL soil to achieve target treatment concentrations in soil for each chemical, respectively, at the required moisture level.

2.4 Treatment Concentrations.

2.4.1 Range Finding Test

Range finding test for Ba, Be, Mn, and Sb were initially conducted using $BaSO_4$, $BeSO_4$, $MnSO_4$, and $Sb_2(SO_4)_3$. Concentrations for Ba and Mn were 100, 500, 1000, 5000 and 10000 mg kg^{-1}. Concentrations for Be and Sb were 1, 10, 100, 500 and 1000 mg kg^{-1}. Additional range finding testing for Ba using BaO, $Ba(NO_3)_2$ and $Ba(C_2H_3O_2)_2$, and for Sb using $Sb_2(C_4H_4O_6)_3$ were done using the same concentrations as for the sulfate salts.

2.4.2 Definitive Tests

Data from the range finding tests were used to determine the respective chemical form with higher toxicity values for *F. candida*, and to determine treatment concentrations for definitive tests. Additional considerations in the selection of the chemical form for definitive toxicity testing was given to chemical solubility in water and the effect each chemical form had on soil pH level. Concentrations selected for definitive tests are shown in Table 1.

Controls included positive (0.05 mg kg^{-1} carbamate), negative (no chemical added) and sulfate ($CaSO_4$). Sulfate controls were based on estimated sulfate amounts in the highest treatment concentrations, and were 7,000 and 35,000 mg kg^{-1} SO_4. Five replicates were used for each treatment concentration and controls.

Table 1. Nominal Ba, Be, Mn, and Sb concentrations selected for definitive toxicity studies with *F. candida*, as determined from range finding tests.

Chemical	Ba	Be	Mn	Sb
First positive concentration tested:				
1	50	10	287	100
2	85	14	500	126
3	144.5	20	695	159
4	245.6	27	966	200
5	417.6	38	1343	252
6	709.9	54	1867	318
7	944	75	2594	400
8	1206.8	105	3606	504
9			5013	

2.5 Weathering/Aging of Amended Soil.

All soil treatment concentrations were subjected to a simulated weathering/aging procedure, which included alternating wetting/drying cycles for 3 weeks prior to commencement of definitive tests. Weathering/aging of test soils was conducted in open plastic bags in the greenhouse. All soil treatments were weighed and adjusted to 60% of the water-holding capacity (WHC) twice each week and then allowed to begin drying. At the end of the weathering/aging period, soil treatments were weighed and brought up to 88% of the WHC prior to initiation of bioassays. A separate study was conducted using Mn as a model chemical to determine if the 3-week duration of weathering/aging procedure was adequate. The duration of this study was 18 weeks. Nominal Mn treatment concentrations included 0, 10, 18, 31, 54, 94, 164, 287, and 503 mg kg^{-1}. Samples from each treatment concentration were analyzed for exchangeable Mn concentrations at 3-week intervals to determine if increase in duration of weathering/aging procedure beyond 3 weeks affects exchangeable Mn concentrations (directly related to bioavailable Mn).

2.6 Chemical Extraction and Analyses.

Soil was analyzed for total metal concentrations following USEPA Method 200.8 (USEPA, 1994) using inductively coupled plasma mass spectrometry (ICP-MS). Additional analysis was done to determine exchangeable Mn fraction. Exchangeable Mn was extracted from soil using 0.05*M* $CaCl_2$ with agitation on a reciprocating shaker for 24 h. All reagents used in extraction of chemicals from soils were either reagent or trace metal grade, and purified water was used throughout the analytical studies. Glassware was washed with phosphate-free detergent followed by rinses with tap water, purified water, nitric acid 1% (v/v) and finally with again with purified water. Analyses of exchangeable Mn concentrations were conducted using a Perkin-Elmer 5100 PC Atomic Absorption Spectrophotometer equipped with an AS-90 autosampler.

2.7 Toxicity Assessment.

The Folsomia Reproduction Test was used to assess the effects of Ba, Be, Mn and Sb on the reproduction of the springtail *Folsomia candida*. The test, referred to as Folsomia Reproduction Test, is an application of the ISO (International Standardization Organization) Soil Quality – Inhibition of Reproduction of Collembola (*Folsomia candida*) by Soil Pollutants, reference number: ISO/FDIS 11267:1998(E). This test is a Chronic/Life-Cycle Assay. The ISO Guideline for this assay was originally developed for use with OECD Artificial Soil (USEPA Standard Artificial Soil); however, we have adapted this methodology for use with natural soils.

2.7.1 Principle of the Test

Ten-to-twelve day-old juveniles are exposed to a range of concentrations of the test chemical added to soil. The test consists of two steps. The first step is a range finding test in which adult survival and total number of juveniles produced are assessed using a limited number of treatment concentrations (typically five) and a reduced number of replicates (three). Based on these results, a series of concentrations are determined for use in the second step, the definitive test. The definitive tests use the same measurement endpoints but are assessed using a greater number of concentrations and replicates. The duration for each test is 4 weeks. The number of adults and juveniles in each treatment concentrations are compared to the numbers in the control(s) to quantify ecotoxicological parameters. These parameters include the bounded No Observed Effect Concentration (NOEC), the bounded Lowest Observed Effect Concentration (LOEC) and the effective concentration that causes an x percent reduction in juvenile numbers, i.e., ECx (e.g., EC_{20}, EC_{50}).

2.7.2 Validity of the Test

Validity criteria are part of Quality Control procedures. Adaptation of the Folsomia Reproduction Test for use with natural soils, included the following performance parameters for the negative controls:

(1) The adult mortality should not exceed 30% at the end of the test;

(2) The average number of juveniles per chamber should reach 80 instars at the end of the 28-day test;

(3) The coefficient of variation for reproduction should not exceed 30%.

2.7.3 Culturing Conditions

The ECBC laboratory culture of *F. candida* was established in 1994 from a stock culture, obtained from the University of Illinois-Chicago, which originated from collembola collected in Kane County, Illinois in 1981. The culture was maintained in culture jars on a mixture of charcoal and plaster of Paris in the dark at 20°C. The springtails were fed baker's yeast and kept moist by routine misting with purified water approximately twice per week. Synchronized cultures were established for the experiments by removing egg clusters from stock cultures and placing them into new jars. Eggs were monitored daily to determine the onset of hatching. Once hatching began, it was allowed to proceed for 2 days, after which juveniles were transferred to new jars. These synchronized juveniles were then held for 10 days, and these procedures provided the 10-12 day-old juveniles used in these studies.

2.7.4 Test Performance

Glass test containers (42 mm ID; 45 mm deep) were rinsed with acetone, tap water and purified water before the test. Twenty grams of prepared soil hydrated to 88% of WHC were added to each test container and 0.05 g of baker's yeast was mixed with soil. The mass of each container including lid and soil was recorded. Each treatment and controls were replicated five times for definitive tests (three for range finding tests). At the initiation of the experiments ten 10-12-day-old juveniles were placed in each test chamber followed by light misting with purified water. A screw lid was placed loosely on each chamber to permit air exchange. The test chambers were randomly placed in an incubator at 20°C with a relative humidity of 90%. During the course of the study, the chambers were misted weekly to maintain soil moisture level.

To terminate a test, purified water (approximately 25 mL) was added to each test chamber to bring the level up to half its volume. After gentle mixing with a spatula, the chamber was examined under a dissecting microscope (15x) for the presence of juveniles and adults. The juveniles and adults that floated to the surface were counted and removed. This procedure was repeated until no other springtails floated to the surface. The chamber was given a final mixing and examined once more to ensure all individuals were counted.

2.8 Data Analysis.

Adult survival and reproduction data were analyzed using nonlinear regression models, described in Stephenson *et al.* (2000). Variances of the residuals were examined to decide whether or not to weight the data, and to select potential models. The Gompertz model had the best fit, regression line was closest to the data points, the variances were the smallest, and the residuals had the best appearance (i.e., most random scattering). That model is:

$$Y = a \times e^{([\log(1-p)]\times[C/\mathrm{ECp}]\wedge b)}$$

where Y is the number of adults or juveniles produced, a is the control response, e is the base of the natural logarithm, p is the percent inhibition/100 (e.g., 0.5 for EC_{50}), C is the exposure concentration in test soil, ECp is the estimate of effect concentration for a specified percent effect, and b is the scale parameter. The ECp parameters used in this study included the metal concentration producing a 20% (EC_{20}) or 50% (EC_{50}) reduction in the measurement endpoint. The EC_{20} parameter based on a reproduction endpoint is the preferred parameter for deriving soil invertebrate Eco-SSL benchmarks. The EC_{50}, more commonly used in the past, and adult survival data were included to enable comparisons of the results produced in this study with results reported by other researchers. The asymptotic standard error (a.s.e.) and 95% confidence intervals (CI) associated with the point estimates were determined.

Analysis of Variance (ANOVA) was used to determine the bounded No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) values for adult survival or juvenile production data (Appendix C). Mean separations were done using Fisher's Least Significant Difference (LSD) pairwise comparison tests. A significance level of $P < 0.05$ was accepted for determining the NOEC and LOEC values. When bounded NOAEC (no observed adverse effect concentration) or bounded LOAEC (lowest observed adverse effect concentration) values were determined, the same statistical methods were used. All analyses except for Sb were done using measured metal concentrations. Statistical analyses were performed using SYSTAT 7.0.1 (SPSS, 1997).

Raw data for range-finding and definitive tests were tabulated and are listed in Appendixes A and B, respectively. Detailed results of statistical analysis of toxicity test data are listed in Appendix D.

3. RESULTS

3.1 Soil Analyses.

Analysis of negative control soil showed that Be concentration in natural SSL soil used in this study was below method detection limit (MDL) of 2.5 mg kg^{-1}. Total Be concentrations in the experimental treatments ranged from 95 to 124% and averaged 107% of nominal (Table 2).

The natural background Mn concentration determined in the negative control treatment was 94 mg kg^{-1}. Total extractable Mn concentrations (in excess of background) in the experimental treatments ranged from 99 to 140% and averaged 111% of nominal (Table 2). Exchangeable Mn fraction expressed as percent of total concentration increased with increasing soil Mn loads (Table 3). There were no trends within any treatment concentration in the amount of exchangeable Mn fraction beyond 3 weeks during the 18-week weathering/aging study. These results confirmed that the 3-week duration for simulated weathering/aging procedure used in to the definitive study design was adequate for the Eco-SSL benchmark development.

Analytical procedures for Sb determination did not confirm agreement with the nominal treatment concentrations. Total Sb treatment concentrations determined using USEPA Method 200.8 ranged from 4 to 21% and averaged 8% of nominal concentration. These results showed that this standard method was not sufficient for total Sb analysis in SSL soil. Additional effort was made in the attempt to improve the analytical procedure. Soils were digested using procedures described in SW-846 Method 3050B (USEPA, 1996). This improved the efficiency of Sb extraction, however it remained relatively low and averaged 58% of nominal concentration added to the soil. For this reason, nominal Sb concentrations were used in determining ecotoxicological parameters for Sb; however because ERA relies on the determination of soil concentrations extracted from soil, toxicity parameters determined from nominal concentrations may have to be adjusted to 58% of their values before determining an Sb Eco-SSL to best conservatively-correspond to the level of Sb extracted from soil at specific levels of Sb toxicity in soil.

The natural background Ba concentration determined in the negative control treatment was 34 mg kg^{-1}. Total Ba concentrations (in excess of background) in the experimental treatments ranged from 89 to 139% and averaged 113% of nominal (Table 2).

Table 2. Results of chemical analyses (following a 3-week weathering/aging procedure) for total Be, Mn, Ba, and Sb, amended individually in SSL soil. Measured concentrations were determined using USEPA Method 200.8 and inductively coupled plasma mass spectrometry (ICP-MS).

Beryllium			Manganese			Barium			Antimony		
Nominal mg kg^{-1}	Measured mg kg^{-1}	Recovery %	Nominal mg kg^{-1}	Measured mg kg^{-1}	Recovery %	Nominal mg kg^{-1}	Measured mg kg^{-1}	Recovery %	Nominal mg kg^{-1}	Measured mg kg^{-1}	Recovery %
0	2.5*		0	94		0	34		0	2.5*	
10	12	95**	287	386	102**	50	83	98**	100	6.4	4
14	18	111	500	633	108	85	110	89	126	4.7	2
20	24	108	695	1067	140	144.5	211	122	159	4.1	1
27	36	124	966	1100	104	245.65	375	139	200	17	16
38	43	107	1343	1667	117	417.61	500	112	252	27	10
54	57	101	1867	2444	126	709.93	800	108	318	5.2	1
75	83	107	2594	2836	106	944	1124	115	400	67	16
105	110	102	3606	3667	99	1206.8	1556	126	504	39	7
			5013	5056	99						

* Method Detection Limit is reported when no metal amount could be determined in negative control soil.

** Percent recovery was determined after correcting metal concentration in treatment soils for the amount present in negative control soil.

The SSL soil pH value of 5.29 was within the range of Eco-SSL's soil matrix of properties that support high bioavailability of cationic metals in natural soils. Soil pH generally decreased with increasing chemical loads but the decrease did not exceed one pH unit (Table 4). In the sulfate control, soil pH decreased by less than 1.0 pH unit in both 7000 and 35000 mg kg^{-1} SO_4^{2-} treatments compared with the negative control.

Table 3. Exchangeable Mn fractions during 18-week weathering/aging study using SSL soil amended with Mn sulfate.

Nominal Mn treatment (mg kg^{-1})	Exchangeable Mn fraction (% of total)						Treatment mean (% of total)
	Week 3	Week 6	Week 9	Week 12	Week 15	Week 18	
0	5.4	4.9	7.3	6.6	6.2	7.7	6.4
10	18.0	16.3	19.9	20.1	16.3	17.8	18.1
18	27.1	25.6	28.7	30.1	23.5	27.9	27.2
31	42.3	37.3	39.1	44.2	38.8	40.5	40.4
54	60.1	52.4	54.9	60.4	48.5	54.5	55.1
94	85.8	75.9	76.0	82.4	65.3	76.7	77.0
164	75.2	63.9	66.7	70.7	56.3	68.9	66.9
287	106.3	93.8	94.3	98.5	82.2	95.8	95.2
503	127.3	99.8	104.7	110.4	101.7	90.3	105.7

Table 4. Summary of soil pH data following a 3-week weathering/aging procedure determined in studies of Be, Mn, Sb, and Ba amended individually in SSL soil.

Ba mg kg^{-1}	pH	Be mg kg^{-1}	pH	Mn Mg kg^{-1}	pH	Sb mg kg^{-1}	pH
0	5.29	0	5.29	0	5.29	0	5.29
50	5.19	10	5.01	287	4.96	100	5.11
85	5.05	14	4.95	500	4.97	126	4.98
144.5	4.99	20	4.89	695	4.90	159	4.94
245.65	4.87	27	4.76	966	4.84	200	4.85
417.61	4.77	38	4.63	1343	4.77	252	4.79
709.93	4.62	54	4.51	1867	4.69	318	4.69
944	4.50	75	4.45	2594	4.65	400	4.69
1206.80	4.47	105	4.29	3606	4.62	504	4.57
				5013	4.56		

3.2 Range Finding Tests.

Barium sulfate ($BaSO_4$) was used to conduct an initial range finding test for Ba toxicity. This test showed that even at the highest concentration tested (10,000 mg kg^{-1}) this essentially insoluble form of Ba did not significantly affect adult survival or juvenile production after 28 days. This necessitated additional range finding tests with alternative forms of Ba. The additional range finding tests were done using Ba soluble in water, including BaO ($LOEC_{juveniles}$ at 500 mg kg^{-1}), $Ba(NO_3)_2$ ($LOEC_{juveniles}$ at 100 mg kg^{-1}), and $Ba(C_2H_3O_2)_2$ ($LOEC_{juveniles}$ at 1,000 mg kg^{-1}). Both BaO and $Ba(C_2H_3O_2)_2$ amendments increased soil pH levels beyond boundaries required by the Eco-SSL guidance for soil parameters supporting high cationic metal bioavailability. Barium oxide increased soil pH to 8.69 and barium acetate increased soil pH to 8.61 at 5,000 mg kg^{-1}, respectively. Soil pH in the barium nitrate test decreased to 4.43 in the 10,000 mg kg^{-1} treatment. Based on the results of these range finding tests barium nitrate was selected for the definitive test, using Ba concentrations shown in Table 1.

A Be range finding test was conducted using $BeSO_4*4H_2O$ (cold water solubility 42.5 g per 100 cc). There was no significant reduction for adults in the concentrations used in this study, however, there was a 100 % reduction in juvenile numbers at the 500 mg kg^{-1} level. Beryllium sulfate hydrate ($BeSO_4*4H_2O$) was retained for the definitive test, using concentrations shown in Table 1.

Manganese sulfate monohydrate ($MnSO_4*H_2O$) was used to conduct a range finding test. Adult survival and juveniles production decreased significantly at 5,000 mg kg^{-1}. There were no juveniles above the 5,000 mg kg^{-1} treatment concentration. Manganese sulfate monohydrate was retained for the definitive test, using concentrations shown in Table 1.

A range finding test for Sb was conducted using $Sb_2(SO_4)_3$. There was no significant reduction for adults in the concentrations used in this study, however, there was a 100% reduction in juvenile numbers at the 1,000 mg kg^{-1} level. A separate study was conducted with antimony D-tartrate $Sb_2(C_4H_4O_6)_3*6H_2O$ to determine if this form was toxic to *F. candida.*

For antimony tartrate, results showed this form was not as toxic as the sulfate form. Antimony sulfate $Sb_2(SO_4)_3$ was retained for the definitive test, using concentrations shown in Table 1.

3.3 Definitive Tests.

Test results complied with validity criteria of the modified protocol for the Folsomia Reproduction Test, accommodating the potentially greater variability in the measurement endpoints when natural soils are used as test media. Definitive tests with aged/weathered SSL soil using the Folsomia Reproduction Tests were conducted to assess the effects of Ba, Be, Mn or Sb on the reproduction of the Collembolan *F. candida*. Ten-to-twelve-day-old *F. candida* were exposed in SSL soil to a range of concentrations for each metal in independent investigations. Measurement endpoints were assessed using 7-8 treatment concentrations determined from the range-finding studies and included the number of surviving adults and juveniles produced after 28 days. All ecotoxicological parameters for Ba, Be, and Mn were estimated using measured chemical concentrations for each treatment level. Ecotoxicological parameters for Sb were estimated using nominal concentrations.

Results of the definitive barium nitrate toxicity testing produced a bounded NOEC for adult survival at the 211 mg kg^{-1} concentration ($P = 0.198$; Figure A-1). Adult survival was significantly ($P \leq 0.0001$) reduced by 30% at the 375 mg kg^{-1} level (LOEC; Table 5). The bounded NOEC for juvenile production was 211 mg kg^{-1} ($P = 0.208$; Figure C-1). The bounded LOEC for juvenile production was 375 mg kg^{-1} ($P \leq 0.0001$). The EC_{20} and EC_{50} values were 165 and 478 mg kg^{-1}, respectively (Table 6; D-1, Appendix D).

Table 5. Summary of ecotoxicological parameters (mg kg^{-1}) for adult *F. candida* survival determined in aged/weathered SSL soil independently amended with Ba, Be, Mn, and Sb using Folsomia Reproduction Test.

Endpoint	Barium	Beryllium	Manganese	Antimony
NOAEC	375	18	2444	100
LOAEC	500	24	2444	126

Beryllium did not significantly affect ($P = 0.603$) adult *F. candida* survival up to the 18 mg kg^{-1}, the NOEC (Table 5; Figure A-2). Adult survival was significantly ($P = 0.007$) reduced at 24 mg kg^{-1} concentration (LOEC). The bounded NOEC for juvenile production was 24 mg kg^{-1} ($P = 0.198$). The bounded LOEC for juvenile production was 36 mg kg^{-1} ($P = 0.030$) (Figure C-2). The EC_{20} and EC_{50} values for juvenile production were 28 and 44 mg kg^{-1}, respectively (Table 6; D-2, Appendix D).

Manganese did not affect ($P = 0.168$) adult springtail survival at the 1667 mg kg^{-1} concentration (NOEC; Figure A-3). Adult survival was significantly reduced ($P \leq 0.0001$) at 2444 mg kg^{-1} (LOEC; Table 5). The bounded NOEC for juvenile production was 1067 mg kg^{-1} ($P = 0.070$; Figure C-3). The bounded LOEC for juvenile production was 1100 mg kg^{-1}

($P = 0.025$; Table 6). No juveniles were produced in 2444 mg kg^{-1} treatment (Figure C-3). The EC_{20} and EC_{50} values for Mn for juvenile production were 1209 and 1663 mg kg^{-1}, respectively (Table 6; D-3, Appendix D).

Antimony did not affect ($P = 0.680$) adult springtails at 100 mg kg^{-1} concentration (NOEC; Figure A-4). Adult survival was significantly reduced ($P = 0.017$) at 126 mg kg^{-1} (LOEC; Table 5). The unbounded LOEC for juvenile production was 100 mg kg^{-1} ($P = 0.045$; Figure C-4), determined using Fisher's least significant difference test. The NOEC for juvenile production was <100 mg kg^{-1}. A bounded NOEC and LOEC values for juvenile production were 100 ($P = 1.0$) and 126 ($P = 0.001$) mg kg^{-1}, respectively, as determined using the Bonferroni pairwise comparison of means. EC_{20} and EC_{50} values for juvenile production for Sb were 81 and 169 mg kg^{-1}, respectively (Table 6; D-4, Appendix D).

Table 6. Summary of ecotoxicological parameters (mg kg^{-1}) for juvenile production determined in aged/weathered SSL soil independently amended with Be, Mn, Sb, and Ba using Folsomia Reproduction Test; parenthetical values are 95% confidence intervals.

Endpoint	Barium	Beryllium	Manganese	Antimony*
NOEC	211	24	1067	<100**
LOEC	375	36	1100	100
EC_{20}	165 (49-281)	28 (18-37)	1209 (979-1438)	81 (46-115)
EC_{50}	478 (325-632)	44 (37-51)	1663 (1491-1834)	169 (135-204)

* Parameters determined using nominal concentrations of Sb in soil.

** This value was derived from Fisher's least significant difference test giving an unbounded NOEC. The more conservative Bonferroni test gave a NOEC of 100 ($P = 1.0$) mg kg^{-1} and a bounded LOEC of 126 mg kg^{-1}.

4. DISCUSSION

Development of screening level benchmarks for Ecological Risk Assessment (ERA) of contaminated soils has become a critical need in recent years (USEPA, 2000). To address this problem, the USEPA in conjunction with stakeholders is developing Eco-SSLs to identify concentrations of chemicals in soil that, when not exceeded, theoretically protective of terrestrial ecosystems within specific soil boundary conditions from unacceptable harmful effects. An extensive review of literature (USEPA, 2000) determined that there was insufficient information for Be, Mn, Sb, and Ba to generate Eco-SSL benchmarks for soil invertebrates. Our toxicity studies were designed to specifically fill this knowledge gap.

The majority of soil toxicity tests that were reported in literature used standard artificial soil with high organic matter content (10%) and near neutral pH. In contrast, we selected SSL soil to meet the criteria for Eco-SSL development, in large part because it has characteristics supporting relatively high bioavailability of cationic metals. In addition, our weathering/aging procedure of the soils loaded with the range of metal concentrations allowed us to more realistically assess the toxicity under conditions more closely resembling the potential toxic effects of Be, Mn, Sb, and Ba in the field.

Definitive toxicity tests conducted with aged/weathered soils amended with test chemicals showed that chemical toxicity order based on all toxicity parameters for juveniles production in tests with *F. candida* was Be > Sb > Ba > Mn (Table 6). However, because ERA relies on the determination of soil concentrations extracted from soil, Sb toxicity parameters determined from nominal concentrations may have to be adjusted to 58% of their values before determining an Sb Eco-SSL in order to best conservatively-correspond to the level of Sb extracted from soil at specific levels of Sb toxicity in soil. However, even when the EC_{20} values for juveniles production for Sb is adjusted by 58% to account for reduced extractability, the relative toxicity order for springtails remains the same. Reproductive endpoints for Ba and Mn tests were more sensitive when compared to adult survival (Tables 5, 6). For Be and Sb, adult survivorship and juveniles production were about equal in their sensitivity. This supports the Eco-SSL requirement of the use of reproductive endpoints for benchmark development. Because this study was designed to produce benchmark data to be used in the development of Eco-SSLs for Be, Mn, Sb, and Ba for soil invertebrates, the test conditions and the resulting data had to meet specific criteria (USEPA, 2000). Thus results from these studies may not directly compare to those of other studies in the literature, since none of them were designed to specifically quantify metal toxicity to soil invertebrates under Eco-SSL conditions of testing using soils that support relatively high bioavailability of cationic metals.

Natural barium concentration in SSL soil of 34 mg kg^{-1} was within the Ba concentrations found in soils (including contaminated sites) at the Aberdeen Proving Ground, which ranged from 9.8 to 1580 mg kg^{-1} (Hlohowskyj *et al.*, 1999). Limited barium ecotoxicological information for soil invertebrates is available from literature. Grace (1990) investigated oral toxicity of barium metaborate to the Eastern Subterranean Termite *Reticulitermes flavipes* (Kollar) in no-choice assays by feeding termite workers for 15 days on filter papers treated with concentrations of 500-40,00 mg kg^{-1} (356-28,472 mg Ba kg^{-1}, recalculated by Kuperman). Results of this study closely correlate with the results of the adult survival (LOEC of 375 mg kg^{-1}) portion of our definitive test; however Grace (1990) reported 19% mortality at the 1780 mg Ba kg^{-1} treatment, but at the highest concentration used in our study (1556 mg kg^{-1}), we obtained a 73% reduction in adults. However, direct comparisons of feeding assays results with soil exposure studies using different species should be treated with caution.

Beryllium is one of the least studied metals regarding its effects on soil invertebrates, although it is considered one of the problem metals of the future (Newland, 1982). It is a component of various fossil fuel types and is increasingly used in aircraft industry, space research, nuclear energy development (Ireland, 1986), X-ray tube, windows manufacturing, and in production of non-sparking tools composed of copper-beryllium alloy (Thorat *et. al.*, 2001). Be concentrations in Aberdeen Proving Ground (APG) soil (including contaminated sites) in the areas adjacent to soil collection ranged from 0.3 to 1.4 mg kg^{-1} (Hlohowskyj *et al.*, 1999). Extensive toxicological studies of Be exposure effects in humans and experimental animals have established that it can cause pulmonary and systemic granulomatous disease known as chronic beryllium disease (Sprince and Kazami, 1980), necrosis and tumors in animals (Witschi, 1971), can inhibit certain enzymes, including alkaline phosphatase (Reiner, 1971), and can inhibit plant and animal growth (Newland, 1982). Ireland (1986) reported increased mortality and growth suppression in a terrestrial snail *Achatina fulica* (Pulmonata) fed 10 μg ml^{-1} Be in the diet containing the sub-optimal calcium concentrations. Among the four chemicals tested in our study, Be was the metal most toxic to springtails based on EC_{20} values.

Natural Mn concentration in SSL soil of 94 mg kg^{-1} was within the range of Mn concentrations reported for soils (including contaminated sites) at the Aberdeen Proving Ground, which ranged from 4.9 to 1140 mg kg^{-1} (Hlohowskyj *et al.*, 1999). Manganese is a required nutrient essential for plants and animals. Manganese was the most previously investigated of the four metals in this study, however none of the previous studies involved invertebrate exposures in natural soils. Reinecke and Reinecke (1996) reported reduction in growth and development (measured as time needed for clitellum development) of *E. fetida* fed with cattle manure spiked with Mn at 151.7 mg kg^{-1}. In our study, we had a 28% reduction in juvenile reproduction at 633 mg kg^{-1}. In a later study, Reinecke and Reinecke (1997) reported damage to spermatozoan structure from treatments containing food spiked with Mn at 61.57 mg kg^{-1}. Nottrot *et al.* (1987) reported no effect on feeding activity and growth of collembolan *Orchesella cincrta* fed with green algae spiked with up to 25 *u*mol Mn g^{-1} dry mass, however that study was conducted on dental plaster. Joosse *et al.* (1983) reported no effect on respiration of woodlice fed with litter containing Mn at 1000 mg kg^{-1} on a porous tile. There was no soil exposure incorporated in that study.

Few studies have investigated Sb concentrations in soil (Cal-Prieto *et al.*, 2001; Crecelius *et al.*, 1974; Kabata-Pendias and Pendias, 1992; van der Voet and de Wolff, 1996). Reported concentrations ranged from 0.17 mg kg^{-1} in organic soils in Norway to 1489 mg kg^{-1} in vicinity of Sb smelter in northeast England (Ainsworth and Cooke, 1991). Concentrations used in our study ranged from 100 to 504 mg kg^{-1}. Antimony concentrations in soil (including contaminated sites) at the Aberdeen Proving Ground in the areas adjacent to the location where the SSL soil was collected ranged from 0.1 to 501 mg kg^{-1} (Hlohowskyj *et al.*, 1999). No information could be found in the available literature on ecotoxicological effects of Sb to soil invertebrates. Developing such information is especially important since input to the soil ecosystems was estimated at 26000 t y^{-1} of Sb (Cal-Prieto *et al.*, 2001). This anthropogenic contribution of Sb is 10-fold higher compared with the Sb emissions from natural sources (ca. 2600 t y^{-1}) reported by Nriagu (1990). Limited data for soil biota was reported by Rafel and Popov (1988) as part of a validation effort for developing the USSR maximum allowable concentrations of Sb in soil. These authors reported 23-52% reduction in seed germination and 26-62% reduction in root growth at 1002 mg kg^{-1} Sb in tests with barley, wheat, radish, pees, and onion. Decrease in ammonia mineralization and nitrate accumulation was observed at Sb concentrations of 52 and 102 mg kg^{-1} in their study. Other measures of soil biological activity were also affected, including decrease in soil enzyme catalase activity and stimulation of soil respiration at 102 mg Sb kg^{-1} (Rafel and Popov, 1988).

Difficulties encountered with the efficiency of extraction of Sb that is aged/weathered in soil prior to analytical determination, using natural SSL amended with Sb, may be symptomatic of a larger problem regarding chemical characterization data during ERA activities at contaminated sites. Low Sb recovery rates using standard USEPA methods suggest that true concentrations of this metal will be underestimated during site characterization efforts. The recovery rates of 8 and 58% determined for Sb aged/weathered in soil in our study, using

USEPA methods 200.8 and 3050B respectively, were below recovery rates of 70 and 88% previously reported for freshly-spiked soils. This clearly indicates that USEPA method 3050B appears better suited to extract aged/weathered Sb from soil, such as that which typically occurs at Superfund and other contaminated sites, and this potential discrepancy in extractability should be corrected for at the time of compilation of a list of contaminants of potential ecological concern (COPEC) in the screening phase of ERA. To use the ecotoxicological parameters from this study, which are based on nominal Sb values, it is recommended that these nominal Sb values be adjusted to 58% of nominal to account for the weathering/aging of Sb in soil (i.e., adjusted to 58% of nominal prior to determining the Eco-SSL). Weathering/aging of Sb in soils typically occurs even more extensively in the field, but simulated weathering/aging provides a conservative estimate of what might otherwise be extractable from field soils. This is especially important given a steep slope of the concentration-response curve for reproductive endpoint determined from the Folsomia Reproduction Test in our study (Figure C-4), which establishes a narrow toxicity threshold range from 81 to 170 mg kg^{-1} based on EC_{20} and EC_{50} estimates (Table 6). The 52 % difference between these two estimates is within the potential recovery error rate of analytical methods used. Disregarding this potential error, especially without adjustment of the Eco-SSL for weathering/aging, can otherwise lead to a removal of Sb from the COPEC list while its extracted concentrations represent field concentrations toxic to relevant ecological receptors. Adjustment of the values of the ecotoxicological parameters determined from nominal concentrations, prior to determination of the Eco-SSL, is properly left to those evaluating benchmarks for Eco-SSL development; however, in these studies an adjustment to 58% of nominal corresponds to the mean recovery rate following 3 weeks of weathering/aging of Sb in soil.

5. CONCLUSIONS

This study has produced ecotoxicological data for barium (Ba), beryllium (Be), manganese (Mn), and antimony (Sb) using an ecologically relevant soil invertebrate species, the springtail *Folsomia candida*. Relative toxicity of the four metals tested in this study was Be > Sb > Ba > Mn, even when nominal Sb values are adjusted by 58% to account for reduced Sb extractability. However, it is strongly recommended that the nominal Sb benchmark values from this study be adjusted to 58% of nominal, in order to account for the weathering/aging of Sb in soil (i.e., adjusted to 58% of nominal prior to determining the Eco-SSL). Study results showed that tests based on reproductive endpoint provide a more sensitive evaluation of effect than adult survival alone, and therefore should be used to set screening criteria. These tests were performed using a natural soil, Sassafras sandy loam. Sassafras sandy loam has relatively low pH, low organic matter, low cation exchange capacity, and high sand content. Such soil characteristics support relatively high bioavailability of cationic metals. Furthermore, aging and weathering of the soil produced a soil microenvironment more similar to field conditions than previous studies where soil invertebrates were exposed immediately following spiking of soil. These study results will be provided to the Ecological Soil Screening Level (Eco-SSL) workgroup for review. Results will undergo quality control review by the Eco-SSL task group before inclusion in the Eco-SSL database, and before being used for developing Ecological Soil Screening Levels (Eco-SSLs) for Be, Mn, Sb, and Ba.

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APPENDIX A

FIGURES FOR ADULT SURVIVORS

Figure A-1. Adult Survivors of *F. candida* exposed to Barium Nitrate in a Sassafras Sandy Loam Soil

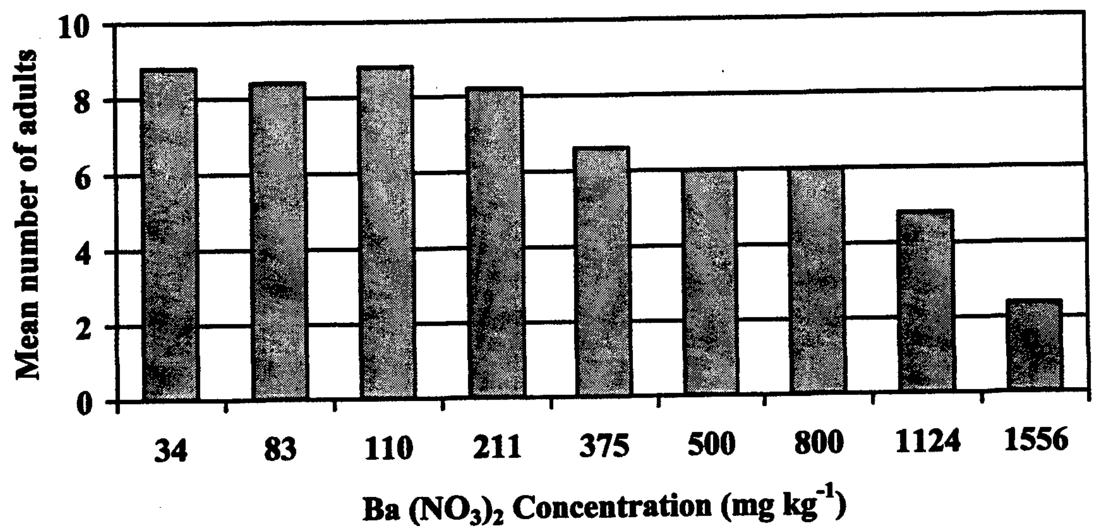


Figure A-2. Adult Survivors of *F. candida* exposed to Beryllium Sulfate in a Sassafras Sandy Loam Soil

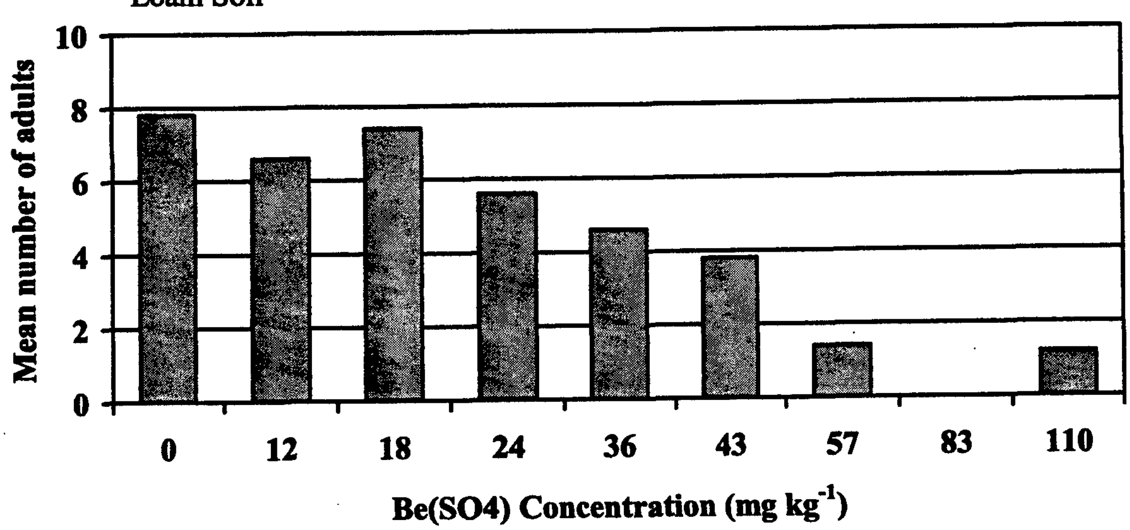


Figure A-3. Adult Survivors of *F. candida* exposed to Manganese Sulfate in a Sassafras Sandy Loam Soil

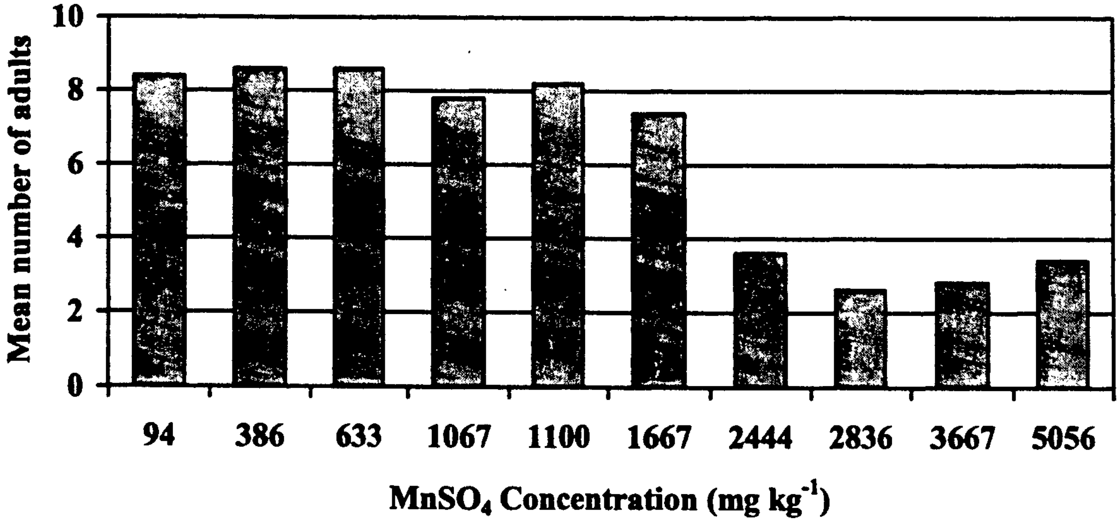
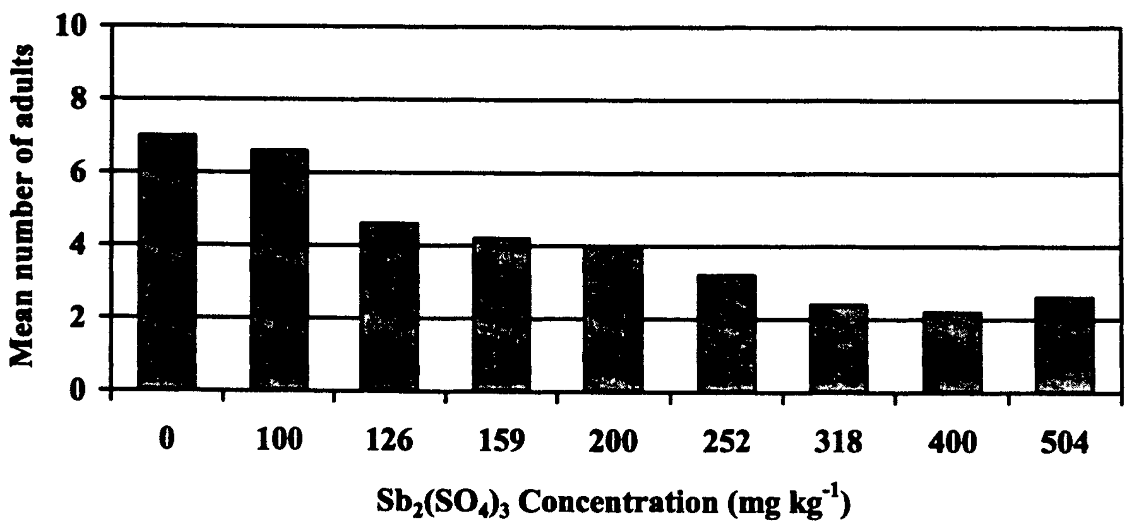


Figure A-4. Adult Survivors of *F. candida* exposed to Antimony Sulfate in a Sassafras Sandy Loam Soil



APPENDIX B

DEFINITIVE TESTS DATA

Table B-1. Barium Nitrate - Life Cycle Testing on Folsomia in SSL Soil -15 Apr 02 – 13 May 02

Treat. mg kg^{-1}	Rep.	Adults	MEAN S.E.	Juv.	MEAN S.E.	% Reduction Adults	% Reduction Juveniles
34	1	9	8.800	178	180.400	0	0
34	2	8	0.374	166	10.211		
34	3	9		213			
34	4	10		191			
34	5	8		154			
83	1	9	8.400	185	178.800	4.545	0.887
83	2	9	0.245	221	12.627		
83	3	8		160			
83	4	8		181			
83	5	8		147			
110	1	9	8.800	162	187.000	0	-3.65
110	2	8	0.374	177	12.518		
110	3	10		234			
110	4	9		189			
110	5	8		173			
211	1	8	8.200	175	172.400	6.82	4.43
211	2	9	0.374	191	9.147		
211	3	9		174			
211	4	8		184			
211	5	7		138			
375	1	7	6.600	128	99.200	25	45.011
375	2	8	0.510	154	19.338		
375	3	5		43			
375	4	6		78			
375	5	7		93			
500	1	6	6.000	83	78.200	31.818	56.652
500	2	5	0.316	55	6.614		
500	3	6		74			
500	4	6		85			
500	5	7		94			
800	1	7	6.000	81	69.600	31.818	61.419
800	2	6	0.316	68	4.142		
800	3	5		63			
800	4	6		77			
800	5	6		59			
1124	1	5	4.800	42	7.638	45.455	95.766
1124	2	5	0.490	61	8.599		
1124	3	6		71			
1124	4	3		28			
1124	5	5		72			
1556	1	2	2.400	1	6.000	72.727	96.674058
1556	2	4	0.510	7	2.933		
1556	3	2		3			
1556	4	1		17			
1556	5	3		2			

Table B-2. Beryllium Sulfate - Life Cycle Testing on Folsomia in SSL Soil -25 Oct 01 – 22 Nov 01

Treat. mg kg^{-1}	Rep.	Adults	MEAN S.E.	Juv.	MEAN S.E.	% Reduction Adults	% Reduction Juveniles
2.5	1	7	7.800	63	85.400	0	0
2.5	2	8	0.200	76	6.816		
2.5	3	8		96			
2.5	4	8		95			
2.5	5	8		97			
12	1	7	6.600	138	101.000	2.5	-18.267
12	2	8	0.510	142	16.787		
12	3	5		72			
12	4	7		93			
12	5	6		60			
18	1	7	7.400	102	91.000	17.5	-6.557
18	2	7	0.245	71	6.116		
18	3	8		94			
18	4	7		104			
18	5	8		84			
24	1	7	5.600	62	70.400	12.5	17.564
24	2	4	0.510	72	8.571		
24	3	5		58			
24	4	6		103			
24	5	6		57			
36	1	7	4.600	68	59.600	17.5	30.210
36	2	5	0.678	48	6.531		
36	3	3		47			
36	4	4		54			
36	5	4		81			
43	1	4	3.800	42	52.000	30	39.110
43	2	5	0.490	67	4.593		
43	3	4		56			
43	4	2		43			
43	5	4		52			
57	1	2	1.400	32	24.400	45	71.429
57	2	0	0.600	2	7.916		
57	3	0		15			
57	4	3		24			
57	5	2		49			
83	1	0	0.000	0	2.600	70	96.956
83	2	0	0.000	9	1.661		
83	3	0		2			
83	4	0		0			
83	5	0		2			
110	1	0	1.200	0	6.400	84.615	92.506
110	2	5	0.970	23	4.501		
110	3	0		0			
110	4	0		0			
110	5	1		9			

Table B-3. Manganese Sulfate - Life Cycle Testing on Folsomia in SSL Soil -16 Apr 02 – 14 May 02

Treat. mg kg^{-1}	Rep.	Adults	MEAN S.E.	Juv.	MEAN S.E.	% Reduction Adults	% Reduction Juveniles
94	1	9	8.400	154	138.000	0	0
94	2	8	0.245	136	8.826		
94	3	9		161			
94	4	8		113			
94	5	8		126			
386	1	9	8.600	141	134.200	-2.381	2.754
386	2	8	0.245	109	9.666		
386	3	8		119			
386	4	9		137			
386	5	9		165			
633	1	9	8.600	176	149.200	-2.381	-8.116
633	2	8	0.245	142	11.972		
633	3	9		155			
633	4	9		166			
633	5	8		107			
1067	1	8	7.800	137	114.200	7.143	17.246
1067	2	8	0.374	122	7.473		
1067	3	9		112			
1067	4	7		92			
1067	5	7		108			
1100	1	7	8.200	77	108.000	2.381	21.739
1100	2	8	0.374	112	9.545		
1100	3	8		98			
1100	4	9		121			
1100	5	9		132			
1667	1	8	7.400	81	78.600	11.905	43.043
1667	2	7	0.510	58	9.811		
1667	3	7		80			
1667	4	9		113			
1667	5	6		61			
2444	1	4	3.600	0	0.000	57.143	100
2444	2	3	0.510	0	0.000		
2444	3	5		0			
2444	4	4		0			
2444	5	2		0			
2836	1	5	2.600	0	0.000	69.048	100
2836	2	2	0.678	0	0.000		
2836	3	1		0			
2836	4	3		0			
2836	5	2		0			

Table B-3. Manganese Sulfate - Life Cycle Testing on Folsomia in SSL Soil -16 Apr 02 – 14 May 02 (Continued)

Treat. mg kg^{-1}	Rep.	Adults	MEAN S.E.	Juv.	MEAN S.E.	% Reduction Adults	% Reduction Juveniles
3667	1	5	2.800	0	0.000	66.667	100
3667	2	3	0.663	0	0.000		
3667	3	2		0			
3667	4	1		0			
3667	5	3		0			
5056	1	4	3.400	0		59.524	100
5056	2	5	0.812	0			
5056	3	5		0			
5056	4	2		0			
5056	5	1		0			

Table B-4. Antimony Sulfate - Life Cycle Testing on Folsomia in SSL Soil -17 Oct 00 – 14 Nov 00

Treat. mg kg^{-1}	Rep.	Adults	MEAN S.E.	Juv.	MEAN S.E.	% Reduction Adults	% Reduction Juveniles
2.5	1	6	7.000	201	207.600	0	0
2.5	2	6	0.632	177	18.739		
2.5	3	8		159			
2.5	4	6		250			
2.5	5	9		251			
100	1	5	6.600	106	163.400	5.714	21.291
100	2	8	0.678	213	22.794		
100	3	8		150			
100	4	5		220			
100	5	7		128			
126	1	6	4.600	117	107.600	34.286	48.170
126	2	6	0.872	135	18.525		
126	3	6		151			
126	4	3		89			
126	5	2		46			
159	1	6	4.200	203	118.800	40	42.775
159	2	5	0.583	119	21.903		
159	3	4		86			
159	4	3		86			
159	5	3		100			
200	1	4	4.000	113	99.400	42.857	52.119
200	2	5	0.316	112	12.444		
200	3	4		77			
200	4	4		131			
200	5	3		64			
252	1	3	3.200	40	45.400	54.286	78.131
252	2	1	0.663	51	11.057		
252	3	5		74			
252	4	4		55			
252	5	3		7			
318	1	2	2.400	24	42.000	65.714	79.769
318	2	2	0.510	49	7.899		
318	3	4		69			
318	4	3		31			
318	5	1		37			
400	1	1	2.200	20	10.800	68.571	94.798
400	2	3	0.374	14	2.782		
400	3	3		5			
400	4	2		6			
400	5	2		9			
504	1	5	2.600	3	1.000	62.857	99.518
504	2	0	1.122	2	0.632		
504	3	0		0			
504	4	5		0			
504	5	3		0			

APPENDIX C

CONCENTRATION-RESPONSE CURVES FOR REPRODUCTION ENDPOINT DETERMINED FROM FRT USING JUVENILE PRODUCTION DATA IN AGED AMENDED SSL SOIL

Figure C-1. Effect of barium on juvenile production by *F. candida* exposed in aged/weathered Sassafras Sandy Loam Soil.

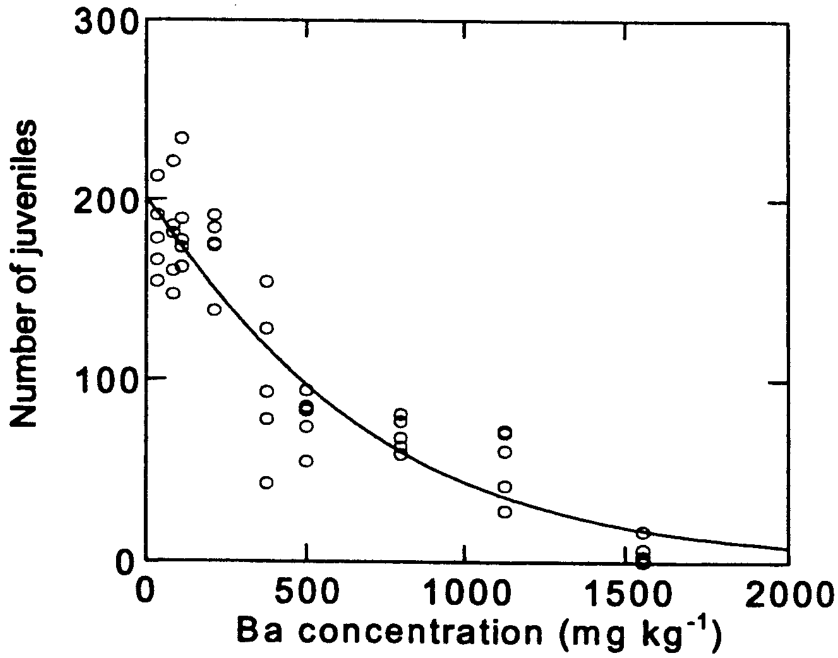


Figure C-2. Effect of beryllium on juvenile production by *F. candida* exposed in aged/weathered Sassafras Sandy Loam Soil.

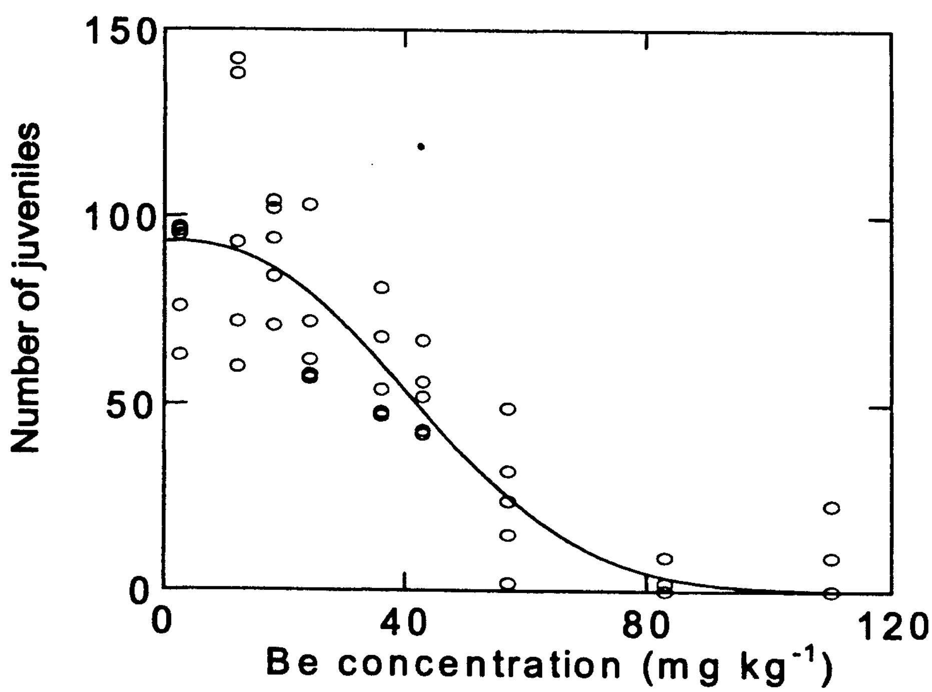


Figure C-3. Effect of manganese on juvenile production by *F. candida* exposed in aged/weathered Sassafras Sandy Loam Soil.

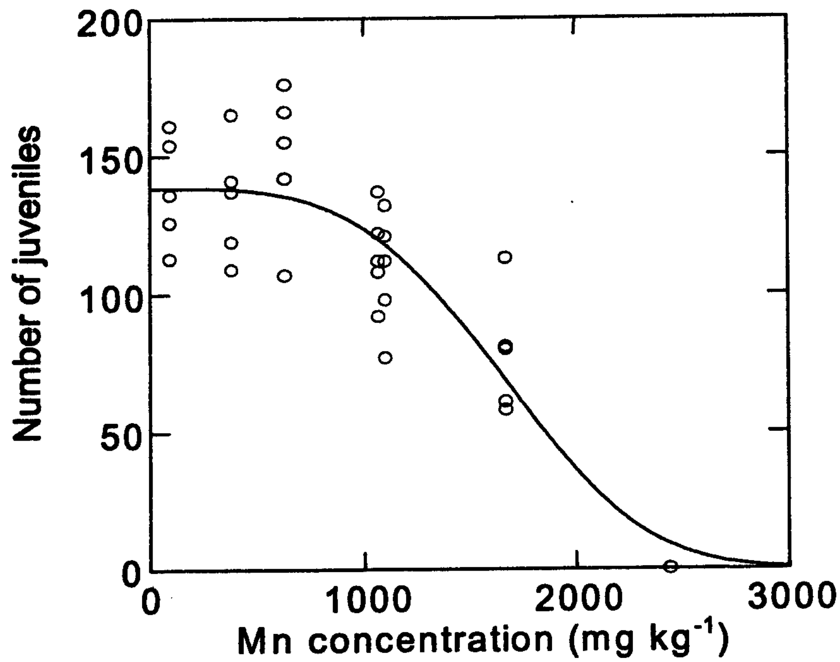
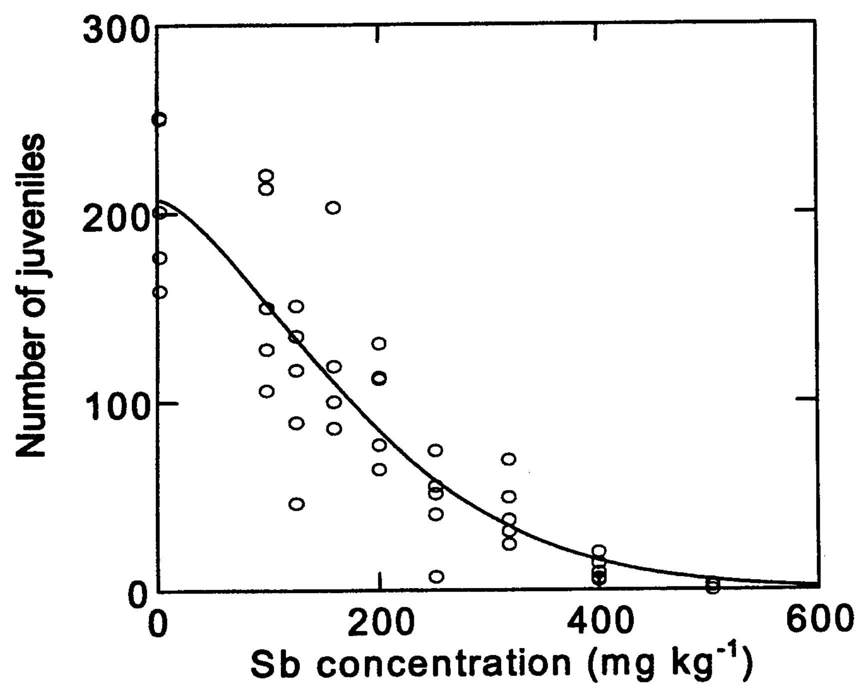


Figure C-4. Effect of antimony on juvenile production by *F. candida* exposed in aged/weathered Sassafras Sandy Loam Soil.



Blank

APPENDIX D

STATISTICAL ANALYSES OF THE DEFINITIVE TESTS DATA

D-1. Statistical analyses of the effect of Ba on *F. candida* in aged SSL soil:

EC$_{50}$ determination for Ba effect on *F. candida* juveniles using Gompertz model.

```
MODEL:

nonlin
print=long
model juveniles=g*exp((log(1-.5))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reBa5FC /resid
estimate/ start = 180, 200, 2 iter=200

45 cases have been saved into a SYSTAT file

 Iteration
 No.       Loss        G            X            B
   0 .173508D+06 .180000D+03 .200000D+03 .200000D+01
   1 .777651D+05 .198689D+03 .333299D+03 .440477D+00
   2 .704762D+05 .175768D+03 .509679D+03 .598421D+00
   3 .415355D+05 .167343D+03 .715351D+03 .127171D+01
   4 .364765D+05 .200331D+03 .405418D+03 .839331D+00
   5 .318157D+05 .189255D+03 .537186D+03 .113927D+01
   6 .310110D+05 .201817D+03 .467014D+03 .102437D+01
   7 .309433D+05 .199732D+03 .483771D+03 .108056D+01
   8 .309386D+05 .201043D+03 .476872D+03 .106149D+01
   9 .309380D+05 .200650D+03 .478980D+03 .106855D+01
  10 .309379D+05 .200800D+03 .478190D+03 .106608D+01
  11 .309379D+05 .200748D+03 .478463D+03 .106696D+01
  12 .309379D+05 .200767D+03 .478366D+03 .106665D+01
  13 .309379D+05 .200760D+03 .478400D+03 .106676D+01
  14 .309379D+05 .200763D+03 .478388D+03 .106672D+01
  15 .309379D+05 .200762D+03 .478392D+03 .106674D+01

Dependent variable is JUVENILES

    Source    Sum-of-Squares    df  Mean-Square
 Regression       754704.071     3   251568.024
   Residual        30937.929    42      736.617

      Total       785642.000    45
Mean corrected    200822.000    44

       Raw  R-square (1-Residual/Total)             =        0.961
Mean corrected R-square (1-Residual/Corrected) =        0.846
          R(observed vs predicted) square       =        0.846

                                                           Wald Confidence Interval
Parameter            Estimate      A.S.E.    Param/ASE        Lower < 95%> Upper
 G                    200.762      14.334       14.006       171.834       229.690
 X                    478.392      76.212        6.277       324.591       632.193
 B                      1.067       0.209        5.111         0.646         1.488

          JUVENILES    JUVENILES
  Case     Observed    Predicted     Residual
      1     178.000      192.640      -14.640
      2     166.000      192.640      -26.640
      3     213.000      192.640       20.360
      4     191.000      192.640       -1.640
      5     154.000      192.640      -38.640
      6     185.000      180.391        4.609
      7     221.000      180.391       40.609
      8     160.000      180.391      -20.391
```

9	181.000	180.391	0.609
10	147.000	180.391	-33.391
11	162.000	173.752	-11.752
12	177.000	173.752	3.248
13	234.000	173.752	60.248
14	189.000	173.752	15.248
15	173.000	173.752	-0.752
16	175.000	150.303	24.697
17	191.000	150.303	40.697
18	174.000	150.303	23.697
19	184.000	150.303	33.697
20	138.000	150.303	-12.303
21	128.000	117.629	10.371
22	154.000	117.629	36.371
23	43.000	117.629	-74.629
24	78.000	117.629	-39.629
25	93.000	117.629	-24.629
26	83.000	97.079	-14.079
27	55.000	97.079	-42.079
28	74.000	97.079	-23.079
29	85.000	97.079	-12.079
30	94.000	97.079	-3.079
31	81.000	60.493	20.507
32	68.000	60.493	7.507
33	63.000	60.493	2.507
34	77.000	60.493	16.507
35	59.000	60.493	-1.493
36	42.000	35.802	6.198
37	61.000	35.802	25.198
38	71.000	35.802	35.198
39	28.000	35.802	-7.802
40	72.000	35.802	36.198
41	1.000	17.514	-16.514
42	7.000	17.514	-10.514
43	3.000	17.514	-14.514
44	17.000	17.514	-0.514
45	0.0	17.514	-17.514

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.872	1.000	
B	-0.822	0.788	1.000

Residuals have been saved.

```
graph
use c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reBa5FC
plot residual*concentr
plot residual*estimate
SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\folsomia\reBa5FC.SYD,
created Wed May 22, 2002 at 08:50:30, contains variables:
 JUVENILES    CONCENTR     ESTIMATE     RESIDUAL
```

```
Stem and Leaf Plot of variable:     RESIDUAL, N = 45
         Minimum:       -74.629
         Lower hinge:       -14.640
         Median:         -0.752
         Upper hinge:        20.360
         Maximum:        60.248
```

```
          -7   4
 * * * Outside Values * * *
          -4   2
          -3   983
          -2   6430
          -1 H 764442210
          -0 M 731100
           0 M 023467
           1   056
           2 H 00345
           3   3566
           4   00
           5
           6   0
```

	RESIDUAL
N of cases	45
Minimum	-74.629
Maximum	60.248
Mean	0.044
Std. Error	3.953
Variance	703.133

EC_{20} determination for Ba effect on *F. candida* juveniles using Gompertz model.

MODEL:

```
nonlin
print=long
model juveniles=g*exp((log(1-.2))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reBa2FC /
resid
estimate/ start = 180, 100, 2 iter=200
```

Iteration

No.	Loss	G	X	B
0	.202973D+06	.180000D+03	.100000D+03	.200000D+01
1	.458309D+05	.193822D+03	.103320D+03	.915779D+00
2	.319389D+05	.191773D+03	.177661D+03	.112875D+01
3	.309541D+05	.201761D+03	.159358D+03	.104185D+01
4	.309395D+05	.200213D+03	.167627D+03	.107471D+01
5	.309381D+05	.200927D+03	.164531D+03	.106379D+01
6	.309380D+05	.200700D+03	.165606D+03	.106776D+01
7	.309379D+05	.200784D+03	.165224D+03	.106637D+01
8	.309379D+05	.200754D+03	.165359D+03	.106686D+01
9	.309379D+05	.200765D+03	.165311D+03	.106669D+01
10	.309379D+05	.200761D+03	.165328D+03	.106675D+01
11	.309379D+05	.200762D+03	.165322D+03	.106673D+01
12	.309379D+05	.200762D+03	.165324D+03	.106673D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	754704.071	3	251568.024
Residual	30937.929	42	736.617
Total	785642.000	45	
Mean corrected	200822.000	44	

```
      Raw  R-square (1-Residual/Total)             =        0.961
Mean corrected R-square (1-Residual/Corrected) =        0.846
         R(observed vs predicted) square         =        0.846
```

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	200.762	14.334	14.006	171.834	229.690
X	165.324	57.459	2.877	49.368	281.281
B	1.067	0.209	5.111	0.646	1.488

Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	178.000	192.640	-14.640
2	166.000	192.640	-26.640
3	213.000	192.640	20.360
4	191.000	192.640	-1.640
5	154.000	192.640	-38.640
6	185.000	180.391	4.609
7	221.000	180.391	40.609
8	160.000	180.391	-20.391
9	181.000	180.391	0.609
10	147.000	180.391	-33.391
11	162.000	173.752	-11.752
12	177.000	173.752	3.248
13	234.000	173.752	60.248
14	189.000	173.752	15.248
15	173.000	173.752	-0.752
16	175.000	150.303	24.697
17	191.000	150.303	40.697
18	174.000	150.303	23.697
19	184.000	150.303	33.697
20	138.000	150.303	-12.303
21	128.000	117.629	10.371
22	154.000	117.629	36.371
23	43.000	117.629	-74.629
24	78.000	117.629	-39.629
25	93.000	117.629	-24.629
26	83.000	97.079	-14.079
27	55.000	97.079	-42.079
28	74.000.	97.079	-23.079
29	85.000	97.079	-12.079
30	94.000	97.079	-3.079
31	81.000	60.493	20.507
32	68.000	60.493	7.507
33	63.000	60.493	2.507
34	77.000	60.493	16.507
35	59.000	60.493	-1.493
36	42.000	35.802	6.198
37	61.000	35.802	25.198
38	71.000	35.802	35.198
39	28.000	35.802	-7.802
40	72.000	35.802	36.198
41	1.000	17.514	-16.514
42	7.000	17.514	-10.514
43	3.000	17.514	-14.514
44	17.000	17.514	-0.514
45	0.0	17.514	-17.514

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.892	1.000	
B	-0.822	0.959	1.000

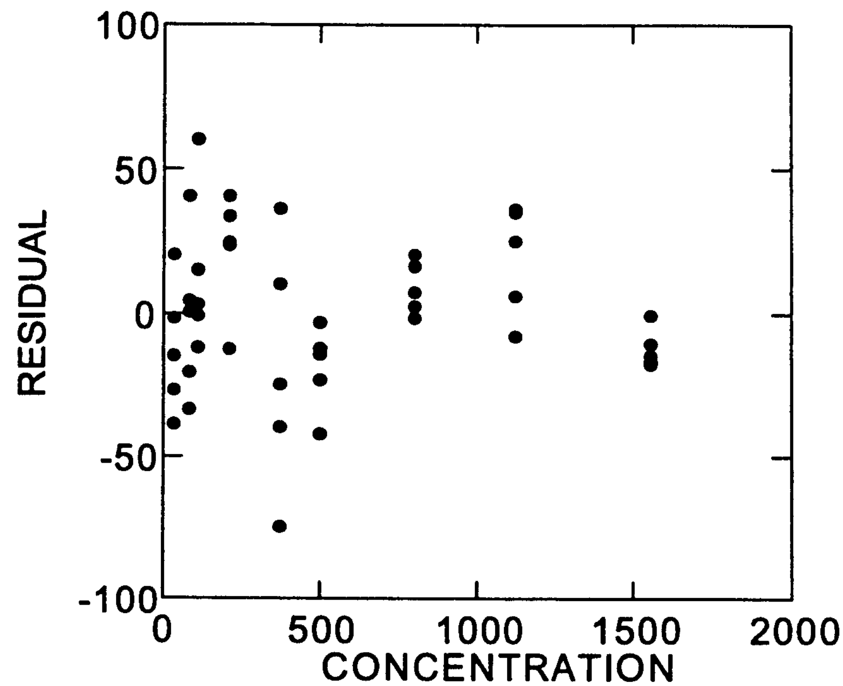
Residuals have been saved.

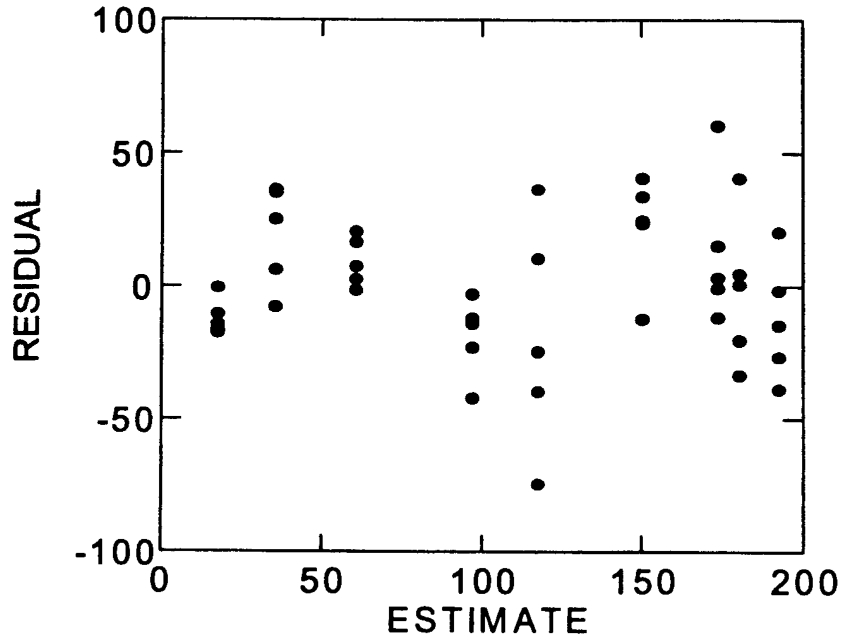
GRAPH MODEL:

```
graph
begin
plot juveniles*concentr / title='', xlab='Ba concentration (mg kg-1)', ylab='Number of
juveniles',
     xmax=2000, xmin=0, ymax=300, ymin=0

fplot y=200.762*exp((log(.5))*(concentr/478.392)^1.067); xmin=0, xmax=2000, xlab=''
ymin=0, ylab='',
     ymax=300   end
```

Residuals for Ba





==========================ANOVA: JUVENILES===========

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (9 levels): 34, 83, 110, 211, 375, 500, 800, 1124, 1556

Dep Var: JUVENILES N: 45 Multiple R: 0.948 Squared multiple R: 0.898

Estimates of effects $B = (X'X)^{-1} X'Y$

		JUVENILES
CONSTANT		114.000
CONCENTR	34	66.400
CONCENTR	83	64.800
CONCENTR	110	73.000
CONCENTR	211	58.400
CONCENTR	375	-14.800
CONCENTR	500	-35.800
CONCENTR	800	-44.400
CONCENTR	1124	-59.200

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTRATION	180374.000	8	22546.750	39.695	0.000
Error	20448.000	36	568.000		

Durbin-Watson D Statistic 2.280
First Order Autocorrelation -0.141

Residuals have been saved.

COL/ ROW	CONCENTRATION
1	34
2	83
3	110
4	211
5	375
6	500
7	800
8	1124
9	1556

Using least squares means.
Post Hoc test of JUVENILES

Using model MSE of 568.000 with 36 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	-1.600	0.0			
3	6.600	8.200	0.0		
4	-8.000	-6.400	-14.600	0.0	
5	-81.200	-79.600	-87.800	-73.200	0.0
6	-102.200	-100.600	-108.800	-94.200	-21.000
7	-110.800	-109.200	-117.400	-102.800	-29.600
8	-125.600	-124.000	-132.200	-117.600	-44.400
9	-174.800	-173.200	-181.400	-166.800	-93.600
	6	7	8	9	
6	0.0				
7	-8.600	0.0			
8	-23.400	-14.800	0.0		
9	-72.600	-64.000	-49.200	0.0	

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.916	1.000			
3	0.664	0.590	1.000		
4	0.599	0.674	0.339	1.000	
5	0.000	0.000	0.000	0.000	1.000
6	0.000	0.000	0.000	0.000	0.172
7	0.000	0.000	0.000	0.000	0.057
8	0.000	0.000	0.000	0.000	0.006
9	0.000	0.000	0.000	0.000	0.000
	6	7	8	9	
6	1.000				
7	0.572	1.000			
8	0.129	0.333	1.000		
9	0.000	0.000	0.002	1.000	

Effects of Barium nitrate on Folsomia in SSL soil - ADULTS Day 28
WED 5/22/02 12:07:22 PM
SYSTAT VERSION 7.0.1

Effects coding used for categorical variables in model.
Categorical values encountered during processing are:
CONCENTRATION (9 levels): 34, 83, 110, 211, 375, 500, 800, 1124, 1556

Dep Var: ADULTS N: 45 Multiple R: 0.930 Squared multiple R: 0.865

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTRATION	185.200	8	23.150	28.938	0.000
Error	28.800	36	0.800		

```
Durbin-Watson D Statistic      2.389
First Order Autocorrelation   -0.201
COL/
ROW CONCENTRATION
    1  34
  2  83
  3  110
  4  211
  5  375
  6  500
  7  800
  8  1124
  9  1556
```

Using least squares means.
Post Hoc test of ADULTS

Using model MSE of 0.800 with 36 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	-0.400	0.0			
3	0.0	0.400	0.0		
4	-0.600	-0.200	-0.600	0.0	
5	-2.200	-1.800	-2.200	-1.600	0.0
6	-2.800	-2.400	-2.800	-2.200	-0.600
7	-2.800	-2.400	-2.800	-2.200	-0.600
8	-4.000	-3.600	-4.000	-3.400	-1.800
9	-6.400	-6.000	-6.400	-5.800	-4.200

	6	7	8	9
6	0.0			
7	0.0	0.0		
8	-1.200	-1.200	0.0	
9	-3.600	-3.600	-2.400	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.484	1.000			
3	1.000	0.484	1.000		
4	0.296	0.726	0.296	1.000	
5	0.000	0.003	0.000	0.008	1.000
6	0.000	0.000	0.000	0.000	0.296
7	0.000	0.000	0.000	0.000	0.296
8	0.000	0.000	0.000	0.000	0.003
9	0.000	0.000	0.000	0.000	0.000

	6	7	8	9
6	1.000			
7	1.000	1.000		
8	0.041	0.041	1.000	
9	0.000	0.000	0.000	1.000

D-2. Statistical analyses of the effect of Be on *F. candida*:

EC_{50} determination for Be effect on *F. candida* juveniles using Gompertz model.

```
SYSTAT VERSION 7.0.1
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MODEL:
nonlin
print=long
model juveniles=g*exp((log(1-.5))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reBe50FC /
resid
estimate/ start = 80, 40, 2 iter=200

45 cases have been saved into a SYSTAT file

 Iteration
 No.        Loss         G              X              B
   0 .204740D+05 .800000D+02 .400000D+02 .200000D+01
   1 .135068D+05 .920525D+02 .454940D+02 .251096D+01
   2 .134358D+05 .932863D+02 .438720D+02 .241299D+01
   3 .134354D+05 .932336D+02 .439185D+02 .243430D+01
   4 .134354D+05 .932489D+02 .439106D+02 .243217D+01
   5 .134354D+05 .932474D+02 .439114D+02 .243241D+01
   6 .134354D+05 .932476D+02 .439113D+02 .243238D+01

Dependent variable is JUVENILES

    Source    Sum-of-Squares    df  Mean-Square
 Regression       186488.648     3    62162.883
   Residual        13435.352    42      319.889

      Total       199924.000    45
Mean corrected     65006.311    44

       Raw  R-square (1-Residual/Total)           =        0.933
Mean corrected R-square (1-Residual/Corrected) =        0.793
          R(observed vs predicted) square         =        0.794

                                                              Wald Confidence Interval
Parameter          Estimate        A.S.E.     Param/ASE        Lower < 95%> Upper
 G                   93.248         5.984        15.583       81.171        105.324
 X                   43.911         3.480        12.620       36.889         50.933
 B                    2.432         0.643         3.784        1.135          3.730

         JUVENILES    JUVENILES
  Case    Observed    Predicted      Residual
     1      63.000       93.187       -30.187
     2      76.000       93.187       -17.187
     3      96.000       93.187         2.813
     4      95.000       93.187         1.813
     5      97.000       93.187         3.813
     6     138.000       90.533        47.467
     7     142.000       90.533        51.467
     8      72.000       90.533       -18.533
     9      93.000       90.533         2.467
    10      60.000       90.533       -30.533
    11     102.000       86.147        15.853
    12      71.000       86.147       -15.147
    13      94.000       86.147         7.853
    14     104.000       86.147        17.853
```

15	84.000	86.147	-2.147
16	62.000	79.503	-17.503
17	72.000	79.503	-7.503
18	58.000	79.503	-21.503
19	103.000	79.503	23.497
20	57.000	79.503	-22.503
21	68.000	60.808	7.192
22	48.000	60.808	-12.808
23	47.000	60.808	-13.808
24	54.000	60.808	-6.808
25	81.000	60.808	20.192
26	42.000	48.259	-6.259
27	67.000	48.259	18.741
28	56.000	48.259	7.741
29	43.000	48.259	-5.259
30	52.000	48.259	3.741
31	32.000	25.225	6.775
32	2.000	25.225	-23.225
33	15.000	25.225	-10.225
34	24.000	25.225	-1.225
35	49.000	25.225	23.775
36	0.0	3.575	-3.575
37	9.000	3.575	5.425
38	2.000	3.575	-1.575
39	0.0	3.575	-3.575
40	2.000	3.575	-1.575
41	0.0	0.144	-0.144
42	23.000	0.144	22.856
43	0.0	0.144	-0.144
44	0.0	0.144	-0.144
45	9.000	0.144	8.856

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.668	1.000	
B	-0.642	0.471	1.000

Residuals have been saved.

EC_{20} determination for Be effect on *F. candida* juveniles using Gompertz model.

MODEL:

```
nonlin
print=long
model juveniles=g*exp((log(1-.2))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reBe20FC/resid
estimate/ start = 80, 20, 2 iter=200
```

Iteration

No.	Loss	G	X	B
0	.250878D+05	.800000D+02	.200000D+02	.200000D+01
1	.135095D+05	.916386D+02	.282611D+02	.240553D+01
2	.134356D+05	.932900D+02	.274533D+02	.242042D+01
3	.134354D+05	.932384D+02	.275655D+02	.243373D+01
4	.134354D+05	.932485D+02	.275543D+02	.243223D+01
5	.134354D+05	.932474D+02	.275555D+02	.243240D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	186488.648	3	62162.883
Residual	13435.352	42	319.889
Total	199924.000	45	
Mean corrected	65006.311	44	

Raw R-square (1-Residual/Total)	=	0.933
Mean corrected R-square (1-Residual/Corrected)	=	0.793
R(observed vs predicted) square	=	0.794

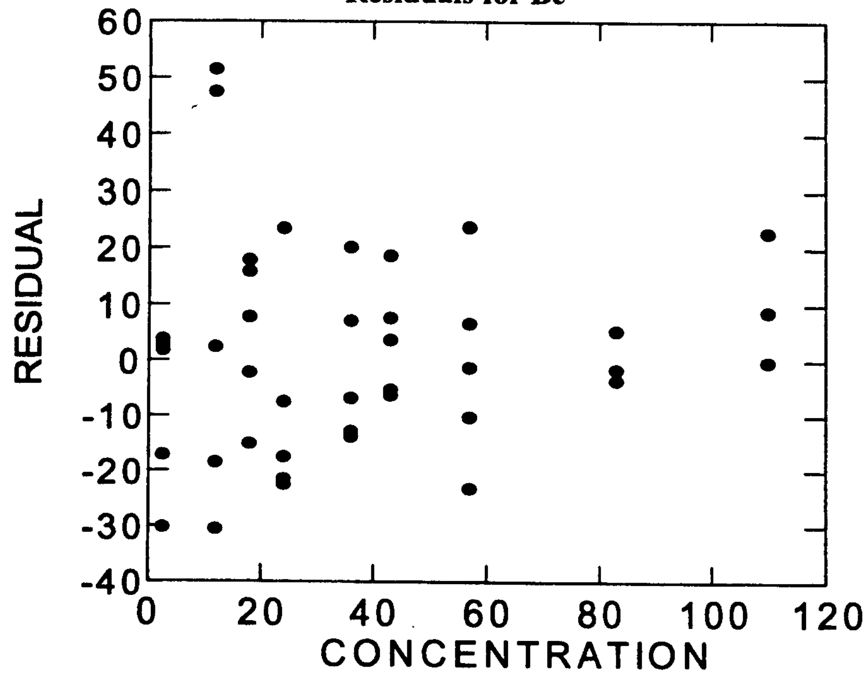
				Wald Confidence Interval	
Parameter	Estimate	A.S.E.	Param/ASE	Lower < 95%>	Upper
G	93.247	5.984	15.583	81.171	105.324
X	27.556	4.823	5.713	17.822	37.289
B	2.432	0.643	3.784	1.135	3.730

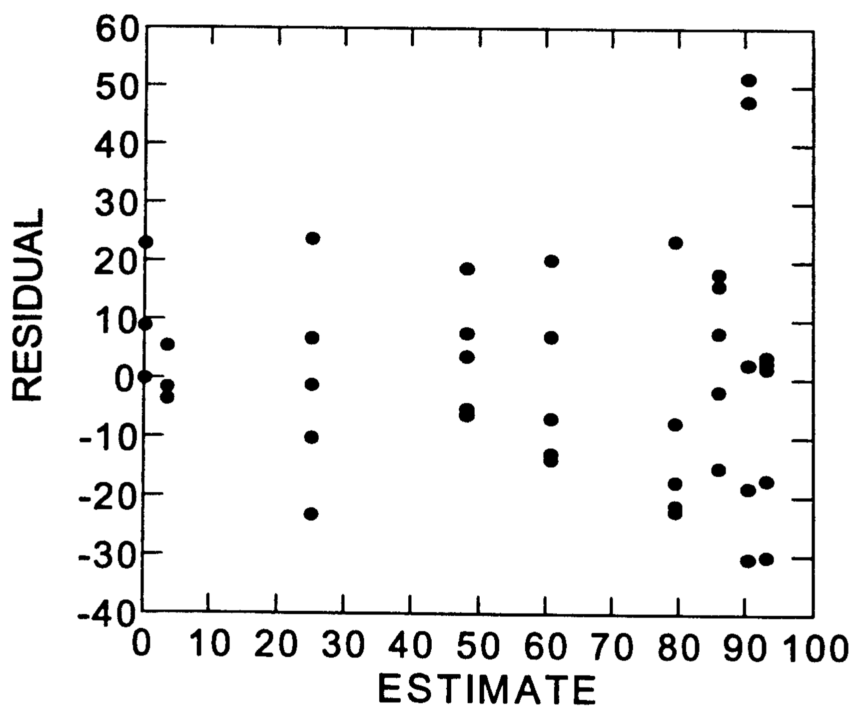
Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	63.000	93.187	-30.187
2	76.000	93.187	-17.187
3	96.000	93.187	2.813
4	95.000	93.187	1.813
5	97.000	93.187	3.813
6	138.000	90.533	47.467
7	142.000	90.533	51.467
8	72.000	90.533	-18.533
9	93.000	90.533	2.467
10	60.000	90.533	-30.533
11	102.000	86.147	15.853
12	71.000	86.147	-15.147
13	94.000	86.147	7.853
14	104.000	86.147	17.853
15	84.000	86.147	-2.147
16	62.000	79.503	-17.503
17	72.000	79.503	-7.503
18	58.000	79.503	-21.503
19	103.000	79.503	23.497
20	57.000	79.503	-22.503
21	68.000	60.808	7.192
22	48.000	60.808	-12.808
23	47.000	60.808	-13.808
24	54.000	60.808	-6.808
25	81.000	60.808	20.192
26	42.000	48.259	-6.259
27	67.000	48.259	18.741
28	56.000	48.259	7.741
29	43.000	48.259	-5.259
30	52.000	48.259	3.741
31	32.000	25.225	6.775
32	2.000	25.225	-23.225
33	15.000	25.225	-10.225
34	24.000	25.225	-1.225
35	49.000	25.225	23.775
36	0.0	3.575	-3.575
37	9.000	3.575	5.425
38	2.000	3.575	-1.575
39	0.0	3.575	-3.575
40	2.000	3.575	-1.575
41	0.0	0.144	-0.144

```
42        23.000          0.144        22.856
43         0.0            0.144        -0.144
44         0.0            0.144        -0.144
45         9.000          0.144         8.856

Asymptotic Correlation Matrix of Parameters

                           G             X             B

G                      1.000
X                     -0.754         1.000
B                     -0.642         0.917         1.000

Residuals have been saved.

MODEL for Residuals:

graph
use c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reBe20FC
plot residual*concentr
plot residual*estimate

SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\folsomia\reBe20FC.SYD,

contains variables:

JUVENILES    CONCENTR     ESTIMATE     RESIDUAL

Stem and Leaf Plot of variable:      RESIDUAL, N = 45
         Minimum:       -30.533
         Lower hinge:       -10.225
         Median:         -0.144
         Upper hinge:         7.741
         Maximum:        51.467


              -3    00
              -2
              -2    321
              -1    8775
              -1 H  320
              -0 H  7665
              -0 M  332111000
               0 M  12233
               0 H  567778
               1
               1    578
               2    0233
      * * * Outside Values * * *
               4    7
               5    1

                         RESIDUAL
 N of cases                  45
 Minimum                -30.533
 Maximum                 51.467
 Mean                     0.602
 Std. Error               2.603
 Standard Dev            17.464
 Variance               304.978
```

```
Graph Model:
graph
begin
plot juveniles*concentr / title='', xlab='Be concentration (mg kg-1)', ylab='Number of
juveniles',
     xmax=120, xmin=0, ymax=150, ymin=0

fplot y=93.248*exp((log(.5))*(concentr/43.911)^2.432); xmin=0, xmax=120, xlab=''
ymin=0, ylab='',
     ymax=150 end
```

Residuals for Be





ANOVA for adults.

THU 5/30/02 9:50:12 AM

SYSTAT VERSION 7.0.1

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTRATION (9 levels): 2.5, 12, 18, 24, 36, 43, 57, 83, 110

Dep Var: ADULTS N: 45 Multiple R: 0.929 Squared multiple R: 0.862

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTRATION	328.400	8	41.050	28.202	0.000
Error	52.400	36	1.456		

*** WARNING ***
Case 42 is an outlier (Studentized Residual = 4.289)

Durbin-Watson D Statistic 2.315
First Order Autocorrelation -0.164

COL/ ROW	CONCENTRATION
1	2.5
2	12
3	18
4	24
5	36
6	43
7	57
8	83
9	110

Using least squares means.
Post Hoc test of ADULTS

Using model MSE of 1.456 with 36 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	-1.200	0.0			
3	-0.400	0.800	0.0		
4	-2.200	-1.000	-1.800	0.0	
5	-3.200	-2.000	-2.800	-1.000	0.0
6	-4.000	-2.800	-3.600	-1.800	-0.800
7	-6.400	-5.200	-6.000	-4.200	-3.200
8	-7.800	-6.600	-7.400	-5.600	-4.600
9	-6.600	-5.400	-6.200	-4.400	-3.400

	6	7	8	9
6	0.0			
7	-2.400	0.0		
8	-3.800	-1.400	0.0	
9	-2.600	-0.200	1.200	0.0

Fisher's Least-Significant-Difference Test.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.125	1.000			
3	0.603	0.301	1.000		
4	0.007	0.198	0.024	1.000	
5	0.000	0.013	0.001	0.198	1.000
6	0.000	0.001	0.000	0.024	0.301
7	0.000	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000
	6	7	8	9	
6	1.000				
7	0.003	1.000			
8	0.000	0.075	1.000		
9	0.002	0.795	0.125	1.000	

ANOVA for juveniles.

Categorical values encountered during processing are:
CONCENTRATION (9 levels): 2.5, 12, 18, 24, 36, 43, 57, 83, 110

Dep Var: JUVENILES N: 45 Multiple R: 0.905 Squared multiple R: 0.819

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTRATION	53235.111	8	6654.389	20.351	0.000
Error	11771.200	36	326.978		

Durbin-Watson D Statistic 2.066
First Order Autocorrelation -0.055

COL/ ROW	CONCENTRATION
1	2.5
2	12
3	18
4	24
5	36
6	43
7	57
8	83
9	110

Using least squares means.
Post Hoc test of JUVENILES

Using model MSE of 326.978 with 36 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	15.600	0.0			
3	5.600	-10.000	0.0		
4	-15.000	-30.600	-20.600	0.0	
5	-25.800	-41.400	-31.400	-10.800	0.0
6	-33.400	-49.000	-39.000	-18.400	-7.600
7	-61.000	-76.600	-66.600	-46.000	-35.200
8	-82.800	-98.400	-88.400	-67.800	-57.000
9	-79.000	-94.600	-84.600	-64.000	-53.200

	6	7	8	9
6	0.0			
7	-27.600	0.0		
8	-49.400	-21.800	0.0	
9	-45.600	-18.000	3.800	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.181	1.000			
3	0.627	0.388	1.000		
4	0.198	0.011	0.080	1.000	
5	0.030	0.001	0.009	0.351	1.000
6	0.006	0.000	0.002	0.116	0.511
7	0.000	0.000	0.000	0.000	0.004
8	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000

	6	7	8	9
6	1.000			
7	0.021	1.000		
8	0.000	0.065	1.000	
9	0.000	0.124	0.742	1.000

D-3. Statistical analyses of the effect of Mn on *F. candida*:

EC_{50} determination for Mn effect on *F. candida* juvenile production in SSL soil.

```
THU 5/23/02 8:18:01 AM

SYSTAT VERSION 7.0.1
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MODEL:

nonlin
print=long
model juveniles=g*exp((log(1-.5))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reMng5FC /
resid
estimate/ start = 130, 1600, 1 iter=200

Graph Model:
graph
begin
plot juveniles*concentr / title='', xlab='Mn concentration (mg kg-1)', ylab='Number of
juveniles',
     xmax=3000, xmin=0, ymax=200, ymin=0

fplot y=138.47*exp((log(.5))*(concentr/1662.573)^3.556); xmin=0, xmax=3000, xlab=''
ymin=0, ylab='',
     ymax=200
end
```

Iteration No.	Loss	G	X	B
0	.479552D+05	.130000D+03	.160000D+04	.100000D+01
1	.463355D+05	.101683D+03	.225735D+04	.317834D+01
2	.404772D+05	.139309D+03	.116896D+04	.266818D+01
3	.188387D+05	.142871D+03	.154811D+04	.200862D+01
4	.143619D+05	.134644D+03	.169626D+04	.326900D+01
5	.137691D+05	.138376D+03	.165038D+04	.363204D+01
6	.137504D+05	.138598D+03	.166518D+04	.349273D+01
7	.137452D+05	.138453D+03	.166061D+04	.357413D+01
8	.137444D+05	.138487D+03	.166320D+04	.354552D+01
9	.137443D+05	.138465D+03	.166225D+04	.355955D+01
10	.137443D+05	.138472D+03	.166270D+04	.355380D+01
11	.137443D+05	.138469D+03	.166251D+04	.355639D+01
12	.137443D+05	.138470D+03	.166260D+04	.355528D+01
13	.137443D+05	.138470D+03	.166256D+04	.355576D+01
14	.137443D+05	.138470D+03	.166258D+04	.355555D+01
15	.137443D+05	.138470D+03	.166257D+04	.355565D+01
16	.137443D+05	.138470D+03	.166257D+04	.355561D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	448402.744	3	149467.581
Residual	13744.256	32	429.508
Total	462147.000	35	
Mean corrected	89594.971	34	

Raw R-square (1-Residual/Total) = 0.970
Mean corrected R-square (1-Residual/Corrected) = 0.847
R(observed vs predicted) square = 0.848

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	138.470	5.769	24.001	126.718	150.222
X	1662.573	84.103	19.768	1491.261	1833.885
B	3.556	0.749	4.744	2.029	5.082

Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	154.000	138.466	15.534
2	136.000	138.466	-2.466
3	161.000	138.466	22.534
4	113.000	138.466	-25.466
5	126.000	138.466	-12.466
6	141.000	137.937	3.063
7	109.000	137.937	-28.937
8	119.000	137.937	-18.937
9	137.000	137.937	-0.937
10	165.000	137.937	27.063
11	176.000	135.406	40.594
12	142.000	135.406	6.594
13	155.000	135.406	19.594
14	166.000	135.406	30.594
15	107.000	135.406	-28.406
16	137.000	119.995	17.005
17	122.000	119.995	2.005
18	112.000	119.995	-7.995
19	92.000	119.995	-27.995
20	108.000	119.995	-11.995
21	77.000	118.045	-41.045
22	112.000	118.045	-6.045

23	98.000	118.045	-20.045
24	121.000	118.045	2.955
25	132.000	118.045	13.955
26	81.000	68.780	12.220
27	58.000	68.780	-10.780
28	80.000	68.780	11.220
29	113.000	68.780	44.220
30	61.000	68.780	-7.780
31	0.0	9.054	-9.054
32	0.0	9.054	-9.054
33	0.0	9.054	-9.054
34	0.0	9.054	-9.054
35	0.0	9.054	-9.054

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.507	1.000	
B	-0.546	0.240	1.000

Residuals have been saved.

RESIDUALS MODEL:

```
graph
use c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reMng5FC
plot residual*concentr
plot residual*estimate
```

SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\folsomia\reMng5FC.SYD,
created Thu May 23, 2002 at 08:29:56, contains variables:
JUVENILES CONCENTR ESTIMATE RESIDUAL

```
Stem and Leaf Plot of variable:     RESIDUAL, N = 35
         Minimum:       -41.045
         Lower hinge:       -11.388
         Median:         -6.045
         Upper hinge:        13.087
         Maximum:        44.220

              -4    1
              -3
              -2    88750
              -1 H  8210
              -0 M  9999977620
               0    2236
               1 H  123579
               2    27
               3    0
               4    04
```

	RESIDUAL
N of cases	35
Minimum	-41.045
Maximum	44.220
Mean	-0.783
Std. Error	3.396
Variance	403.611

EC_{20} determination for Mn effect on *F. candida* juvenile production in SSL soil.

```
MODEL:

nonlin
print=long
model juveniles=g*exp((log(1-.2))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reMng2FC /
resid
estimate/ start = 130, 700, 1 iter=200

 Iteration
 No.      Loss         G            X            B
   0 .444534D+05 .130000D+03 .700000D+03 .100000D+01
   1 .391886D+05 .118734D+03 .149504D+04 .226717D+01
   2 .175840D+05 .129259D+03 .112562D+04 .286113D+01
   3 .137532D+05 .138326D+03 .122188D+04 .360459D+01
   4 .137445D+05 .138373D+03 .121154D+04 .357439D+01
   5 .137443D+05 .138460D+03 .120899D+04 .355413D+01
   6 .137443D+05 .138465D+03 .120891D+04 .355742D+01
   7 .137443D+05 .138470D+03 .120874D+04 .355502D+01
   8 .137443D+05 .138469D+03 .120878D+04 .355591D+01
   9 .137443D+05 .138470D+03 .120875D+04 .355550D+01
  10 .137443D+05 .138470D+03 .120876D+04 .355567D+01
  11 .137443D+05 .138470D+03 .120876D+04 .355559D+01

Dependent variable is JUVENILES

     Source    Sum-of-Squares    df  Mean-Square
 Regression        448402.744     3   149467.581
   Residual         13744.256    32      429.508

      Total        462147.000    35
Mean corrected      89594.971    34

       Raw  R-square (1-Residual/Total)            =        0.970
Mean corrected R-square (1-Residual/Corrected) =        0.847
          R(observed vs predicted) square      =        0.848

                                                           Wald Confidence Interval
Parameter            Estimate        A.S.E.     Param/ASE       Lower < 95%> Upper
 G                    138.470         5.769        24.001     126.718       150.222
 X                   1208.759       112.758        10.720     979.078      1438.440
 B                      3.556         0.749         4.744       2.029         5.082

           JUVENILES     JUVENILES
   Case     Observed     Predicted      Residual
      1      154.000       138.466        15.534
      2      136.000       138.466        -2.466
      3      161.000       138.466        22.534
      4      113.000       138.466       -25.466
      5      126.000       138.466       -12.466
      6      141.000       137.937         3.063
      7      109.000       137.937       -28.937
      8      119.000       137.937       -18.937
      9      137.000       137.937        -0.937
     10      165.000       137.937        27.063
     11      176.000       135.406        40.594
     12      142.000       135.406         6.594
     13      155.000       135.406        19.594
     14      166.000       135.406        30.594
     15      107.000       135.406       -28.406
     16      137.000       119.995        17.005
```

17	122.000	119.995	2.005
18	112.000	119.995	-7.995
19	92.000	119.995	-27.995
20	108.000	119.995	-11.995
21	77.000	118.045	-41.045
22	112.000	118.045	-6.045
23	98.000	118.045	-20.045
24	121.000	118.045	2.955
25	132.000	118.045	13.955
26	81.000	68.780	12.220
27	58.000	68.780	-10.780
28	80.000	68.780	11.220
29	113.000	68.780	44.220
30	61.000	68.780	-7.780
31	0.0	9.054	-9.054
32	0.0	9.054	-9.054
33	0.0	9.054	-9.054
34	0.0	9.054	-9.054
35	0.0	9.054	-9.054

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.668	1.000	
B	-0.546	0.850	1.000

Residuals have been saved.

ANOVA for juveniles.

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTRATIONS (7 levels): 94, 386, 633, 1067, 1100, 1667, 2444

Dep Var: JUVENILES N: 35 Multiple R: 0.936 Squared multiple R: 0.875

Estimates of effects $B = (X'X)^{-1} X'Y$

		JUVENILES
CONSTANT		103.171
CONCENTR	94	34.829
CONCENTR	386	31.029
CONCENTR	633	46.029
CONCENTR	1067	11.029
CONCENTR	1100	4.829
CONCENTR	1667	-24.571

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTRATION	78437.371	6	13072.895	32.806	0.000
Error	11157.600	28	398.486		

Durbin-Watson D Statistic 2.189
First Order Autocorrelation -0.106

COL/ ROW	CONCENTRATION
1	94
2	386
3	633
4	1067
5	1100
6	1667
7	2444

Using least squares means.
Post Hoc test of JUVENILES

Using model MSE of 398.486 with 28 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	-3.800	0.0			
3	11.200	15.000	0.0		
4	-23.800	-20.000	-35.000	0.0	
5	-30.000	-26.200	-41.200	-6.200	0.0
6	-59.400	-55.600	-70.600	-35.600	-29.400
7	-138.000	-134.200	-149.200	-114.200	-108.000

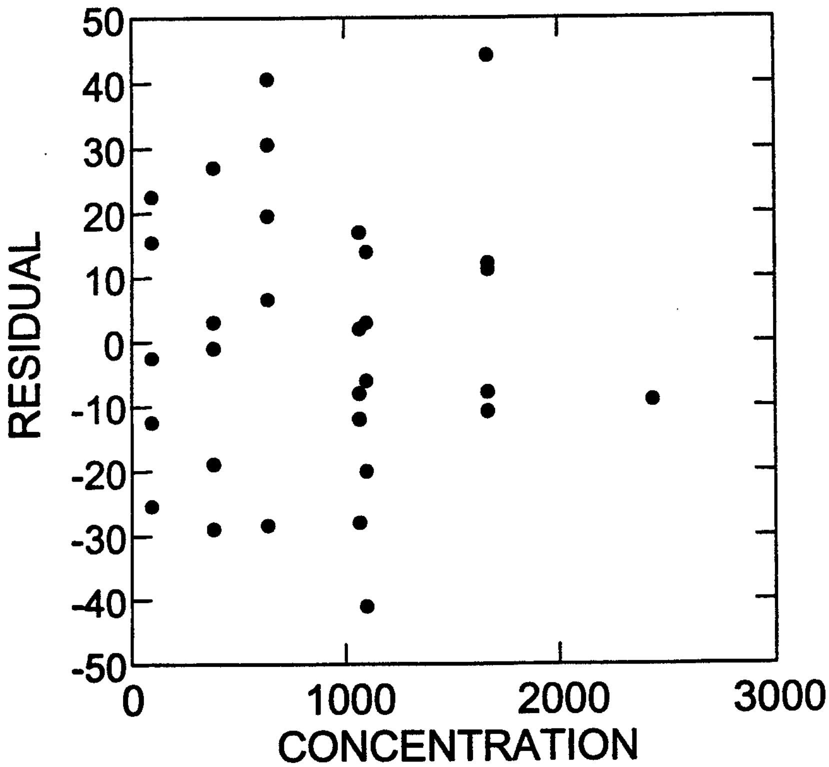
	6	7
6	0.0	
7	-78.600	0.0

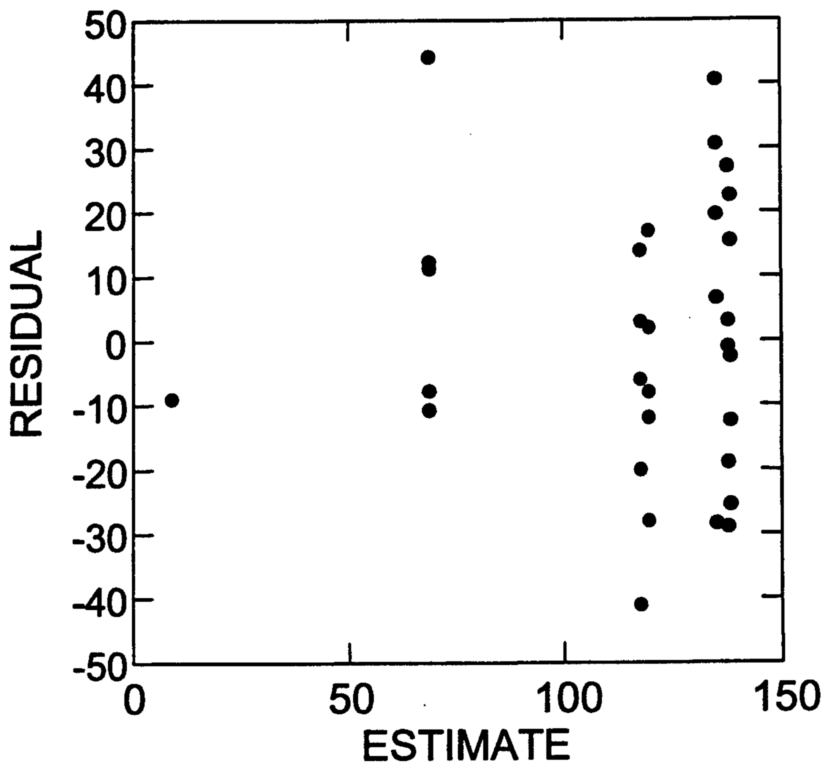
Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.766	1.000			
3	0.383	0.245	1.000		
4	0.070	0.124	0.010	1.000	
5	0.025	0.047	0.003	0.627	1.000
6	0.000	0.000	0.000	0.009	0.027
7	0.000	0.000	0.000	0.000	0.000

	6	7
6	1.000	
7	0.000	1.000

Residuals for Mn





EC_{50} determination for Mn effect on *F. candida* adult survival using Gompertz model.

MODEL:

```
nonlin
print=long
model adults=g*exp((log(1-.5))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reMng5FA /
resid
estimate/ start = 8, 2000, 1 iter=200
```

Graph Model:

```
graph
begin
plot juveniles*concentr / title='', xlab='Mn concentration (mg kg-1)', ylab='Number of
juveniles',
     xmax=3000, xmin=0, ymax=200, ymin=0

fplot y=138.47*exp((log(.5))*(concentr/1662.573)^3.556); xmin=0, xmax=3000, xlab=''
ymin=0, ylab='',
     ymax=200
end
```

Iteration

No.	Loss	G	X	B
0	.217794D+03	.800000D+01	.200000D+04	.100000D+01
1	.107818D+03	.847095D+01	.306282D+04	.152522D+01
2	.105668D+03	.942479D+01	.243891D+04	.103902D+01
3	.102113D+03	.881019D+01	.280464D+04	.141141D+01
4	.100544D+03	.931800D+01	.251500D+04	.124803D+01
5	.100160D+03	.910466D+01	.263100D+04	.138796D+01
6	.100072D+03	.924253D+01	.255609D+04	.133034D+01
7	.100050D+03	.918031D+01	.258751D+04	.136757D+01
8	.100045D+03	.921440D+01	.256922D+04	.135103D+01
9	.100044D+03	.919779D+01	.257771D+04	.136024D+01
10	.100043D+03	.920640D+01	.257317D+04	.135581D+01
11	.100043D+03	.920208D+01	.257540D+04	.135812D+01
12	.100043D+03	.920428D+01	.257425D+04	.135697D+01
13	.100043D+03	.920317D+01	.257483D+04	.135756D+01
14	.100043D+03	.920374D+01	.257453D+04	.135726D+01
15	.100043D+03	.920345D+01	.257468D+04	.135741D+01
16	.100043D+03	.920360D+01	.257460D+04	.135734D+01
17	.100043D+03	.920352D+01	.257464D+04	.135738D+01
18	.100043D+03	.920356D+01	.257462D+04	.135736D+01

Dependent variable is ADULTS

Source	Sum-of-Squares	df	Mean-Square
Regression	2152.957	3	717.652
Residual	100.043	47	2.129
Total	2253.000	50	
Mean corrected	368.020	49	

Raw R-square (1-Residual/Total)	=	0.956
Mean corrected R-square (1-Residual/Corrected)	=	0.728
R(observed vs predicted) square	=	0.730

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	9.204	0.565	16.296	8.067	10.340
X	2574.624	282.666	9.108	2005.974	3143.274
B	1.357	0.282	4.808	0.789	1.925

Case	ADULTS Observed	ADULTS Predicted	Residual
1	9.000	9.132	-0.132
2	8.000	9.132	-1.132
3	9.000	9.132	-0.132
4	8.000	9.132	-1.132
5	8.000	9.132	-1.132
6	9.000	8.731	0.269
7	8.000	8.731	-0.731
8	8.000	8.731	-0.731
9	9.000	8.731	0.269
10	9.000	8.731	0.269
11	9.000	8.301	0.699
12	8.000	8.301	-0.301
13	9.000	8.301	0.699
14	9.000	8.301	0.699
15	8.000	8.301	-0.301
16	8.000	7.463	0.537
17	8.000	7.463	0.537
18	9.000	7.463	1.537
19	7.000	7.463	-0.463
20	7.000	7.463	-0.463
21	7.000	7.397	-0.397
22	8.000	7.397	0.603
23	8.000	7.397	0.603
24	9.000	7.397	1.603
25	9.000	7.397	1.603
26	8.000	6.267	1.733
27	7.000	6.267	0.733
28	7.000	6.267	0.733
29	9.000	6.267	2.733
30	6.000	6.267	-0.267
31	4.000	4.825	-0.825
32	3.000	4.825	-1.825
33	5.000	4.825	0.175
34	4.000	4.825	-0.825
35	2.000	4.825	-2.825
36	5.000	4.175	0.825
37	2.000	4.175	-2.175
38	1.000	4.175	-3.175
39	3.000	4.175	-1.175
40	2.000	4.175	-2.175
41	5.000	3.002	1.998
42	3.000	3.002	-0.002
43	2.000	3.002	-1.002
44	1.000	3.002	-2.002
45	3.000	3.002	-0.002
46	4.000	1.628	2.372
47	5.000	1.628	3.372
48	5.000	1.628	3.372
49	2.000	1.628	0.372
50	1.000	1.628	-0.628

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.792	1.000	
B	-0.751	0.620	1.000

```
Residuals have been saved.
RESIDUALS MODEL:
graph
use c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reMng5FA
plot residual*concentr
plot residual*estimate

SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\folsomia\reMng5FA.SYD,
created Thu May 23, 2002 at 09:17:34, contains variables:
 ADULTS        CONCENTR      ESTIMATE      RESIDUAL


Stem and Leaf Plot of variable:     RESIDUAL, N = 50
         Minimum:          -3.175
         Lower hinge:          -0.825
         Median:           -0.002
         Upper hinge:           0.699
         Maximum:           3.372

              -3    1
      * * * Outside Values * * *
              -2    8
              -2    110
              -1    8
              -1    11110
              -0 H 88776
              -0 M 4433321100
               0 M 12223
               0 H 5566666778
               1
               1    56679
               2    3
               2    7
      * * * Outside Values * * *
               3    33


                         RESIDUAL
  N of cases                 50
  Minimum                -3.175
  Maximum                 3.372
  Mean                    0.048
  Std. Error              0.202
  Variance                2.039


Graph Model:
graph
begin
plot adults*concentr / title='', xlab='Mn concentration (mg kg-1)', ylab='Number of
adults',
     xmax=6000, xmin=0, ymax=10, ymin=0

fplot y=9.204*exp((log(.5))*(concentr/2574.624)^1.357); xmin=0, xmax=6000, xlab=''
ymin=0, ylab='',
     ymax=10
end
```

EC_{20} determination for Mn effect on *F. candida* adult survival using Gompertz model.

MODEL:

```
nonlin
print=long
model adults=g*exp((log(1-.2))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reMng2FA/resid
estimate/ start = 8, 1600, 1 iter=200
```

Iteration No.	Loss	G	X	B
0	.166194D+03	.800000D+01	.160000D+04	.100000D+01
1	.116940D+03	.871649D+01	.913607D+03	.105844D+01
2	.101821D+03	.895185D+01	.125492D+04	.140609D+01
3	.100546D+03	.931770D+01	.101342D+04	.125055D+01
4	.100168D+03	.910364D+01	.116480D+04	.138943D+01
5	.100075D+03	.924421D+01	.108892D+04	.132905D+01
6	.100051D+03	.917900D+01	.113044D+04	.136814D+01
7	.100045D+03	.921508D+01	.110990D+04	.135059D+01
8	.100044D+03	.919741D+01	.112057D+04	.136042D+01
9	.100043D+03	.920658D+01	.111522D+04	.135570D+01
10	.100043D+03	.920199D+01	.111796D+04	.135817D+01
11	.100043D+03	.920433D+01	.111658D+04	.135694D+01
12	.100043D+03	.920315D+01	.111728D+04	.135757D+01
13	.100043D+03	.920375D+01	.111692D+04	.135726D+01
14	.100043D+03	.920344D+01	.111710D+04	.135742D+01
15	.100043D+03	.920360D+01	.111701D+04	.135734D+01
16	.100043D+03	.920352D+01	.111706D+04	.135738D+01
17	.100043D+03	.920356D+01	.111703D+04	.135736D+01
18	.100043D+03	.920354D+01	.111705D+04	.135737D+01

Dependent variable is ADULTS

Source	Sum-of-Squares	df	Mean-Square
Regression	2152.957	3	717.652
Residual	100.043	47	2.129
Total	2253.000	50	
Mean corrected	368.020	49	

Raw R-square (1-Residual/Total) = 0.956
Mean corrected R-square (1-Residual/Corrected) = 0.728
R(observed vs predicted) square = 0.730

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	9.204	0.565	16.296	8.067	10.340
X	1117.047	286.668	3.897	540.346	1693.748
B	1.357	0.282	4.808	0.789	1.925

Case	ADULTS Observed	ADULTS Predicted	Residual
1	9.000	9.132	-0.132
2	8.000	9.132	-1.132
3	9.000	9.132	-0.132
4	8.000	9.132	-1.132
5	8.000	9.132	-1.132
6	9.000	8.731	0.269
7	8.000	8.731	-0.731
8	8.000	8.731	-0.731
9	9.000	8.731	0.269
10	9.000	8.731	0.269
11	9.000	8.301	0.699
12	8.000	8.301	-0.301
13	9.000	8.301	0.699

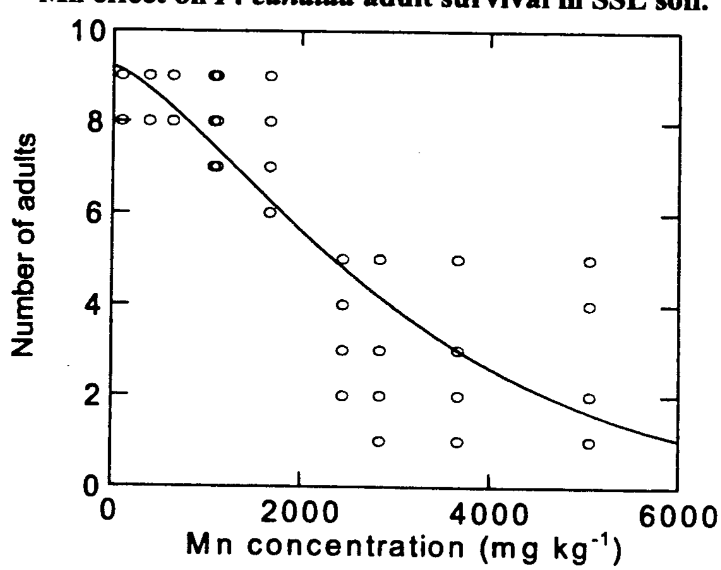
14	9.000	8.301	0.699
15	8.000	8.301	-0.301
16	8.000	7.463	0.537
17	8.000	7.463	0.537
18	9.000	7.463	1.537
19	7.000	7.463	-0.463
20	7.000	7.463	-0.463
21	7.000	7.397	-0.397
22	8.000	7.397	0.603
23	8.000	7.397	0.603
24	9.000	7.397	1.603
25	9.000	7.397	1.603
26	8.000	6.267	1.733
27	7.000	6.267	0.733
28	7.000	6.267	0.733
29	9.000	6.267	2.733
30	6.000	6.267	-0.267
31	4.000	4.825	-0.825
32	3.000	4.825	-1.825
33	5.000	4.825	0.175
34	4.000	4.825	-0.825
35	2.000	4.825	-2.825
36	5.000	4.175	0.825
37	2.000	4.175	-2.175
38	1.000	4.175	-3.175
39	3.000	4.175	-1.175
40	2.000	4.175	-2.175
41	5.000	3.002	1.998
42	3.000	3.002	-0.002
43	2.000	3.002	-1.002
44	1.000	3.002	-2.002
45	3.000	3.002	-0.002
46	4.000	1.628	2.372
47	5.000	1.628	3.372
48	5.000	1.628	3.372
49	2.000	1.628	0.372
50	1.000	1.628	-0.628

Asymptotic Correlation Matrix of Parameters

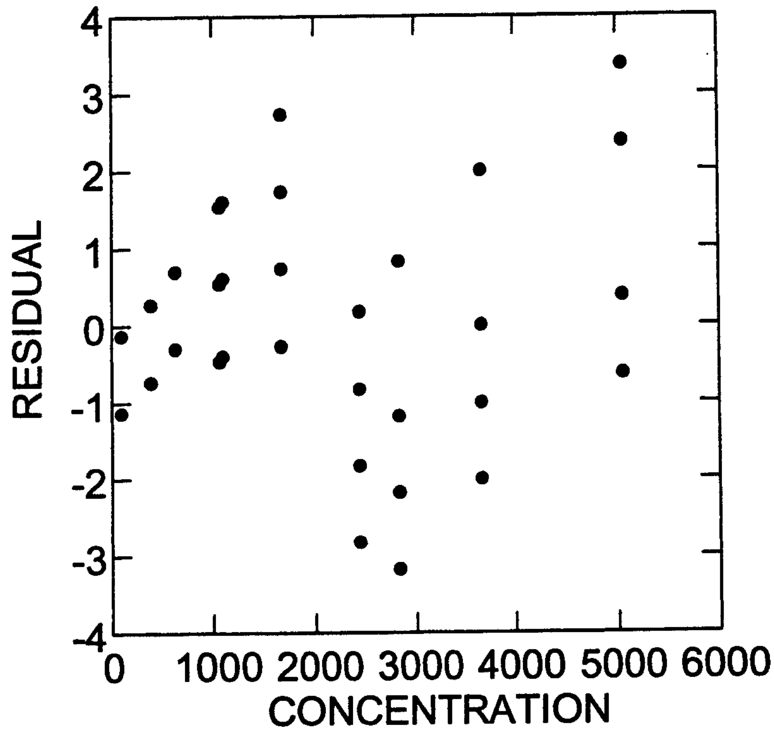
	G	X	B
G	1.000		
X	-0.847	1.000	
B	-0.751	0.942	1.000

Residuals have been saved.

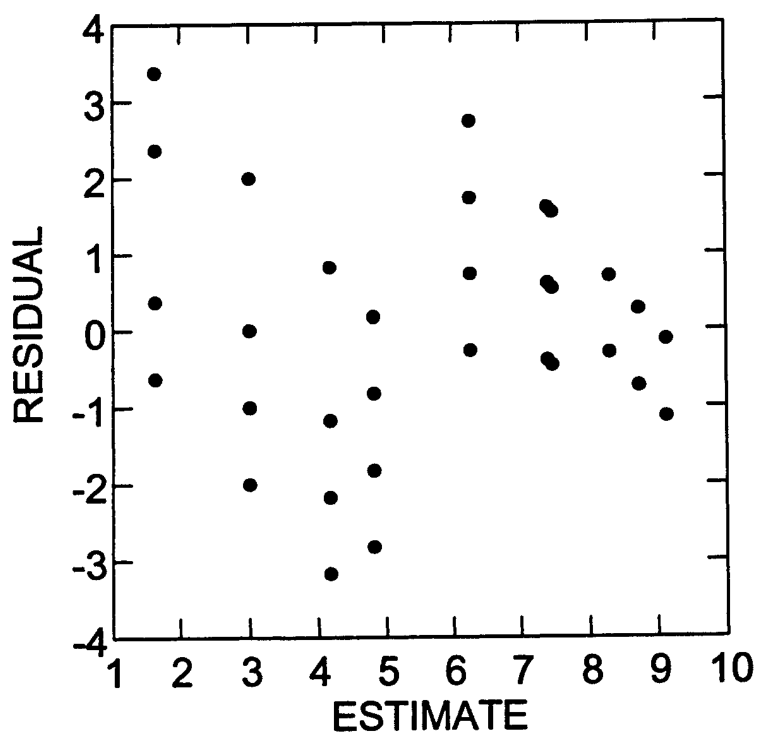
Mn effect on *F. candida* adult survival in SSL soil.



Residuals



RESIDUAL
4
3
2
1
0
-1
-2
-3
-4
0
1000
2000
3000
4000
5000
6000
CONCENTRATION



RESIDUAL
4
3
2
1
0
-1
-2
-3
-4
1
2
3
4
5
6
7
8
9
10
ESTIMATE

ANOVA for adults.

```
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTRATION (10 levels): 94, 386, 633, 1067, 1100, 1667, 2444, 2836, 3667, 5056

Dep Var: ADULTS   N: 50   Multiple R: 0.928   Squared multiple R: 0.862


                                       -1
Estimates of effects   B = (X'X)   X'Y

                                        ADULTS

     CONSTANT                            6.140
     CONCENTR     94                     2.260
     CONCENTR     386                    2.460
     CONCENTR     633                    2.460
     CONCENTR     1067                   1.660
     CONCENTR     1100                   2.060
     CONCENTR     1667                   1.260
     CONCENTR     2444                  -2.540
     CONCENTR     2836                  -3.540
     CONCENTR     3667                  -3.340


                              Analysis of Variance

Source              Sum-of-Squares   df   Mean-Square     F-ratio       P

CONCENTRATION              317.220    9        35.247      27.753     0.000

Error                       50.800   40         1.270

-------------------------------------------------------------------------------

Durbin-Watson D Statistic      2.042
First Order Autocorrelation   -0.081
COL/
ROW CONCENTRATION
  1  94
  2  386
  3  633
  4  1067
  5  1100
  6  1667
  7  2444
  8  2836
  9  3667
 10  5056
Using least squares means.
Post Hoc test of ADULTS
-------------------------------------------------------------------------------
```

Using model MSE of 1.270 with 40 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	0.200	0.0			
3	0.200	0.0	0.0		
4	-0.600	-0.800	-0.800	0.0	
5	-0.200	-0.400	-0.400	0.400	0.0
6	-1.000	-1.200	-1.200	-0.400	-0.800
7	-4.800	-5.000	-5.000	-4.200	-4.600
8	-5.800	-6.000	-6.000	-5.200	-5.600
9	-5.600	-5.800	-5.800	-5.000	-5.400
10	-5.000	-5.200	-5.200	-4.400	-4.800
	6	7	8	9	10
6	0.0				
7	-3.800	0.0			
8	-4.800	-1.000	0.0		
9	-4.600	-0.800	0.200	0.0	
10	-4.000	-0.200	0.800	0.600	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.780	1.000			
3	0.780	1.000	1.000		
4	0.405	0.268	0.268	1.000	
5	0.780	0.578	0.578	0.578	1.000
6	0.168	0.100	0.100	0.578	0.268
7	0.000	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000
10	0.000	0.000	0.000	0.000	0.000
	6	7	8	9	10
6	1.000				
7	0.000	1.000			
8	0.000	0.168	1.000		
9	0.000	0.268	0.780	1.000	
10	0.000	0.780	0.268	0.405	1.000

D-4. Statistical analyses of the effect of Sb on *F. candida*:

EC_{50} determination for Sb effect on *F. candida* juvenile production in SSL soil.

```
MODEL:

nonlin
print=long
model juveniles=g*exp((log(1-.5))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reSbg5FC /
resid
estimate/ start = 200, 160, liter=200

 Iteration
 No.       Loss        G            X            B
   0 .582349D+05 .200000D+03 .160000D+03 .100000D+01
   1 .479593D+05 .205308D+03 .175408D+03 .149295D+01
   2 .475590D+05 .207264D+03 .169061D+03 .153214D+01
   3 .475573D+05 .207352D+03 .169269D+03 .152743D+01
   4 .475573D+05 .207346D+03 .169276D+03 .152805D+01
   5 .475573D+05 .207346D+03 .169276D+03 .152800D+01

Dependent variable is JUVENILES

    Source    Sum-of-Squares   df  Mean-Square
 Regression       539826.720    3   179942.240
   Residual        47557.280   42     1132.316

      Total       587384.000   45
Mean corrected    235375.111   44

      Raw  R-square (1-Residual/Total)             =        0.919
Mean corrected R-square (1-Residual/Corrected) =        0.798
         R(observed vs predicted) square         =        0.798

                                                             Wald Confidence Interval
Parameter          Estimate       A.S.E.     Param/ASE         Lower < 95%> Upper
 G                  207.346       14.936        13.883       177.205        237.487
 X                  169.276       17.239         9.820       134.487        204.065
 B                    1.528        0.270         5.653         0.983          2.073

          JUVENILES    JUVENILES
  Case     Observed    Predicted      Residual
     1      201.000      207.117        -6.117
     2      177.000      207.117       -30.117
     3      159.000      207.117       -48.117
     4      250.000      207.117        42.883
     5      251.000      207.117        43.883
     6      106.000      152.059       -46.059
     7      213.000      152.059        60.941
     8      150.000      152.059        -2.059
     9      220.000      152.059        67.941
    10      128.000      152.059       -24.059
    11      117.000      133.343       -16.343
    12      135.000      133.343         1.657
    13      151.000      133.343        17.657
    14       89.000      133.343       -44.343
    15       46.000      133.343       -87.343
    16      203.000      110.442        92.558
    17      119.000      110.442         8.558
    18       86.000      110.442       -24.442
```

19	86.000	110.442	-24.442
20	100.000	110.442	-10.442
21	113.000	84.778	28.222
22	112.000	84.778	27.222
23	77.000	84.778	-7.778
24	131.000	84.778	46.222
25	64.000	84.778	-20.778
26	40.000	58.047	-18.047
27	51.000	58.047	-7.047
28	74.000	58.047	15.953
29	55.000	58.047	-3.047
30	7.000	58.047	-51.047
31	24.000	33.712	-9.712
32	49.000	33.712	15.288
33	69.000	33.712	35.288
34	31.000	33.712	-2.712
35	37.000	33.712	3.288
36	20.000	15.724	4.276
37	14.000	15.724	-1.724
38	5.000	15.724	-10.724
39	6.000	15.724	-9.724
40	9.000	15.724	-6.724
41	3.000	5.274	-2.274
42	2.000	5.274	-3.274
43	0.0	5.274	-5.274
44	0.0	5.274	-5.274
45	0.0	5.274	-5.274

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.770	1.000	
B	-0.524	0.624	1.000

Residuals have been saved.

```
RESIDUALS MODEL:
graph
use c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reSbg5FC
plot residual*concentr
plot residual*estimate
```

SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\folsomia\reSbg5FC.SYD,
contains variables:

JUVENILES CONCENTR ESTIMATE RESIDUAL

```
Stem and Leaf Plot of variable:     RESIDUAL, N = 45
         Minimum:       -87.343
         Lower hinge:       -16.343
         Median:        -5.274
         Upper hinge:        15.288
         Maximum:        92.558

              -8   7
      * * * Outside Values * * *
              -5   1
              -4   864
              -3   0
              -2   4440
              -1 H 8600
              -0 M 997766555332221
               0   1348
```

```
            1 H 557
            2   78
            3   5
            4   236
            5
            6   0
      * * * Outside Values * * *
            6   7
            9   2
```

	RESIDUAL
N of cases	45
Minimum	-87.343
Maximum	92.558
Mean	-0.500
Std. Error	4.900
Variance	1080.592

```
Graph Model:
graph
begin
plot juveniles*concentr / title='', xlab='Sb concentration (mg kg-1)', ylab='Number of
juveniles',
     xmax=600, xmin=0, ymax=300, ymin=0

fplot y=207.346*exp((log(.5))*(concentr/169.276)^1.528); xmin=0, xmax=600, xlab=''
ymin=0, ylab='',
     ymax=300
end
```

EC_{20} determination for Sb effect on *F. candida* juvenile production in SSL soil.

```
MODEL:

nonlin
print=long
model juveniles=g*exp((log(1-.2))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reSbg2FC /
resid
estimate/ start = 200, 80, 1 iter=200
```

Iteration No.	Loss	G	X	B
0	.980923D+05	.200000D+03	.800000D+02	.100000D+01
1	.499584D+05	.209244D+03	.651120D+02	.134381D+01
2	.475611D+05	.207292D+03	.799927D+02	.151582D+01
3	.475573D+05	.207345D+03	.806482D+02	.152867D+01
4	.475573D+05	.207346D+03	.806190D+02	.152795D+01
5	.475573D+05	.207346D+03	.806213D+02	.152801D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	539826.720	3	179942.240
Residual	47557.280	42	1132.316
Total	587384.000	45	
Mean corrected	235375.111	44	

Raw R-square (1-Residual/Total)	=	0.919
Mean corrected R-square (1-Residual/Corrected)	=	0.798
R(observed vs predicted) square	=	0.798

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%> Upper	
G	207.346	14.936	13.883	177.204	237.487
X	80.621	16.963	4.753	46.388	114.855
B	1.528	0.270	5.653	0.983	2.074

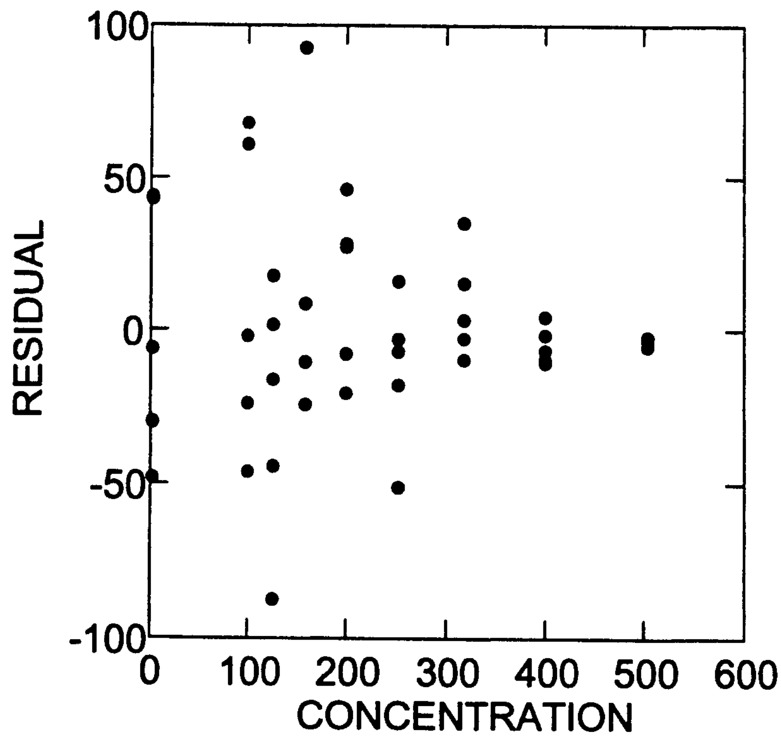
Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	201.000	207.117	-6.117
2	177.000	207.117	-30.117
3	159.000	207.117	-48.117
4	250.000	207.117	42.883
5	251.000	207.117	43.883
6	106.000	152.059	-46.059
7	213.000	152.059	60.941
8	150.000	152.059	-2.059
9	220.000	152.059	67.941
10	128.000	152.059	-24.059
11	117.000	133.343	-16.343
12	135.000	133.343	1.657
13	151.000	133.343	17.657
14	89.000	133.343	-44.343
15	46.000	133.343	-87.343
16	203.000	110.442	92.558
17	119.000	110.442	8.558
18	86.000	110.442	-24.442
19	86.000	110.442	-24.442
20	100.000	110.442	-10.442
21	113.000	84.778	28.222
22	112.000	84.778	27.222
23	77.000	84.778	-7.778
24	131.000	84.778	46.222
25	64.000	84.778	-20.778
26	40.000	58.047	-18.047
27	51.000	58.047	-7.047
28	74.000	58.047	15.953
29	55.000	58.047	-3.047
30	7.000	58.047	-51.047
31	24.000	33.712	-9.712
32	49.000	33.712	15.288
33	69.000	33.712	35.288
34	31.000	33.712	-2.712
35	37.000	33.712	3.288
36	20.000	15.724	4.276
37	14.000	15.724	-1.724
38	5.000	15.724	-10.724
39	6.000	15.724	-9.724
40	9.000	15.724	-6.724
41	3.000	5.274	-2.274
42	2.000	5.274	-3.274
43	0.0	5.274	-5.274
44	0.0	5.274	-5.274
45	0.0	5.274	-5.274

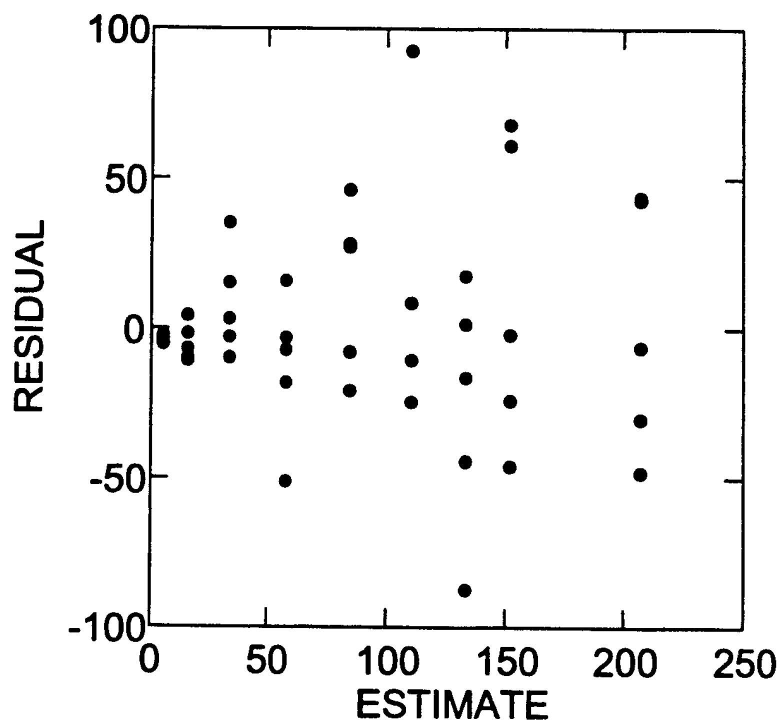
Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.699	1.000	
B	-0.524	0.926	1.000

Residuals have been saved.

Residuals for Sb





Effects of Antimony on *F. candida* adult survival

EC_{50} determination for Sb effect on *F. candida* adult survival in SSL soil.

```
MODEL:

nonlin
print=long
model adults=g*exp((log(1-.5))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reSbg5FA /
resid
estimate/ start = 7, 200, 1 iter=200
```

Iteration No.	Loss	G	X	B
0	.109193D+03	.700000D+01	.200000D+03	.100000D+01
1	.977310D+02	.727277D+01	.234187D+03	.962604D+00
2	.976604D+02	.728937D+01	.237110D+03	.948089D+00
3	.976601D+02	.728661D+01	.237452D+03	.947416D+00
4	.976601D+02	.728664D+01	.237457D+03	.947242D+00
5	.976601D+02	.728658D+01	.237462D+03	.947255D+00

Dependent variable is ADULTS

Source	Sum-of-Squares	df	Mean-Square
Regression	860.340	3	286.780
Residual	97.660	42	2.325
Total	958.000	45	
Mean corrected	205.644	44	

Raw R-square (1-Residual/Total)	=	0.898
Mean corrected R-square (1-Residual/Corrected)	=	0.525
R(observed vs predicted) square	=	0.525

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	7.287	0.740	9.851	5.794	8.779
X	237.462	49.132	4.833	138.310	336.615
B	0.947	0.271	3.490	0.399	1.495

Case	ADULTS Observed	ADULTS Predicted	Residual
1	6.000	7.219	-1.219
2	6.000	7.219	-1.219
3	8.000	7.219	0.781
4	6.000	7.219	-1.219
5	9.000	7.219	1.781
6	5.000	5.368	-0.368
7	8.000	5.368	2.632
8	8.000	5.368	2.632
9	5.000	5.368	-0.368
10	7.000	5.368	1.632
11	6.000	4.982	1.018
12	6.000	4.982	1.018
13	6.000	4.982	1.018
14	3.000	4.982	-1.982
15	2.000	4.982	-2.982
16	6.000	4.536	1.464
17	5.000	4.536	0.464

18	4.000	4.536	-0.536
19	3.000	4.536	-1.536
20	3.000	4.536	-1.536
21	4.000	4.043	-0.043
22	5.000	4.043	0.957
23	4.000	4.043	-0.043
24	4.000	4.043	-0.043
25	3.000	4.043	-1.043
26	3.000	3.500	-0.500
27	1.000	3.500	-2.500
28	5.000	3.500	1.500
29	4.000	3.500	0.500
30	3.000	3.500	-0.500
31	2.000	2.921	-0.921
32	2.000	2.921	-0.921
33	4.000	2.921	1.079
34	3.000	2.921	0.079
35	1.000	2.921	-1.921
36	1.000	2.340	-1.340
37	3.000	2.340	0.660
38	3.000	2.340	0.660
39	2.000	2.340	-0.340
40	2.000	2.340	-0.340
41	5.000	1.772	3.228
42	0.0	1.772	-1.772
43	0.0	1.772	-1.772
44	5.000	1.772	3.228
45	3.000	1.772	1.228

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.846	1.000	
B	-0.622	0.593	1.000

Residuals have been saved.

```
RESIDUALS MODEL:
graph
use c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reSbg5FA
plot residual*concentr
plot residual*estimate
```

```
SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\folsomia\reSbg5FA.SYD,
created Wed May 29, 2002 at 13:26:28, contains variables:
 ADULTS       CONCENTR      ESTIMATE      RESIDUAL
```

```
Stem and Leaf Plot of variable:     RESIDUAL, N = 45
         Minimum:          -2.982
         Lower hinge:          -1.219
         Median:           -0.043
         Upper hinge:           1.018
         Maximum:           3.228

              -2    9
              -2    4
              -1    997755
              -1 H  32220
              -0    995
              -0 M  443333000
               0 M  04
               0    56679
```

```
                  1 H 000024
                  1   567
                  2
                  2   66
                  3   22

                        RESIDUAL
  N of cases                  45
  Minimum                 -2.982
  Maximum                  3.228
  Mean                     0.013
  Std. Error               0.222
  Variance                 2.219
```

```
Graph Model:
graph
begin
plot juveniles*concentr / title='', xlab='Sb concentration (mg kg-1)', ylab='Number of
adults',
     xmax=600, xmin=0, ymax=10, ymin=0

fplot y=7.287*exp((log(.5))*(concentr/237.462)^0.947); xmin=0, xmax=600, xlab=''
ymin=0, ylab='',
     ymax=10
end
```

<u>EC_{20} determination for Sb effect on *F. candida* adult survival in SSL soil.</u>

```
MODEL:

nonlin
print=long
model adults=g*exp((log(1-.2))*(concentr/x)^b)
save c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\folsomia\reSbg2FA /
resid
estimate/ start = 7, 100, 1 iter=200

Iteration
 No.        Loss        G            X            B
   0 .105528D+03 .700000D+01 .100000D+03 .100000D+01
   1 .100011D+03 .735457D+01 .556621D+02 .857354D+00
   2 .977095D+02 .721734D+01 .738315D+02 .966226D+00
   3 .976606D+02 .729422D+01 .713345D+02 .944319D+00
   4 .976602D+02 .728504D+01 .718624D+02 .947917D+00
   5 .976601D+02 .728692D+01 .717498D+02 .947111D+00
   6 .976601D+02 .728652D+01 .717739D+02 .947281D+00
   7 .976601D+02 .728660D+01 .717688D+02 .947244D+00
   8 .976601D+02 .728658D+01 .717699D+02 .947252D+00

Dependent variable is ADULTS

    Source    Sum-of-Squares    df  Mean-Square
 Regression          860.340     3      286.780
   Residual           97.660    42        2.325

      Total          958.000    45
Mean corrected       205.644    44

       Raw  R-square (1-Residual/Total)           =         0.898
Mean corrected R-square (1-Residual/Corrected) =         0.525
          R(observed vs predicted) square         =         0.525
```

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	7.287	0.740	9.851	5.794	8.779
X	71.770	35.486	2.022	0.157	143.383
B	0.947	0.271	3.490	0.399	1.495

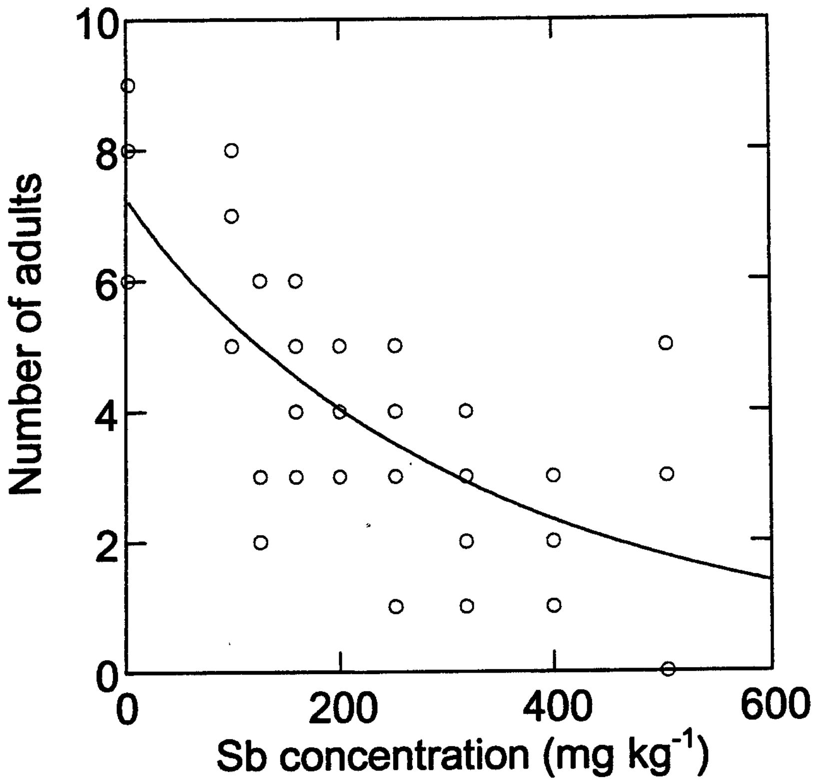
Case	ADULTS Observed	ADULTS Predicted	Residual
1	6.000	7.219	-1.219
2	6.000	7.219	-1.219
3	8.000	7.219	0.781
4	6.000	7.219	-1.219
5	9.000	7.219	1.781
6	5.000	5.368	-0.368
7	8.000	5.368	2.632
8	8.000	5.368	2.632
9	5.000	5.368	-0.368
10	7.000	5.368	1.632
11	6.000	4.982	1.018
12	6.000	4.982	1.018
13	6.000	4.982	1.018
14	3.000	4.982	-1.982
15	2.000	4.982	-2.982
16	6.000	4.536	1.464
17	5.000	4.536	0.464
18	4.000	4.536	-0.536
19	3.000	4.536	-1.536
20	3.000	4.536	-1.536
21	4.000	4.043	-0.043
22	5.000	4.043	0.957
23	4.000	4.043	-0.043
24	4.000	4.043	-0.043
25	3.000	4.043	-1.043
26	3.000	3.500	-0.500
27	1.000	3.500	-2.500
28	5.000	3.500	1.500
29	4.000	3.500	0.500
30	3.000	3.500	-0.500
31	2.000	2.921	-0.921
32	2.000	2.921	-0.921
33	4.000	2.921	1.079
34	3.000	2.921	0.079
35	1.000	2.921	-1.921
36	1.000	2.340	-1.340
37	3.000	2.340	0.660
38	3.000	2.340	0.660
39	2.000	2.340	-0.340
40	2.000	2.340	-0.340
41	5.000	1.772	3.228
42	0.0	1.772	-1.772
43	0.0	1.772	-1.772
44	5.000	1.772	3.228
45	3.000	1.772	1.228

Asymptotic Correlation Matrix of Parameters

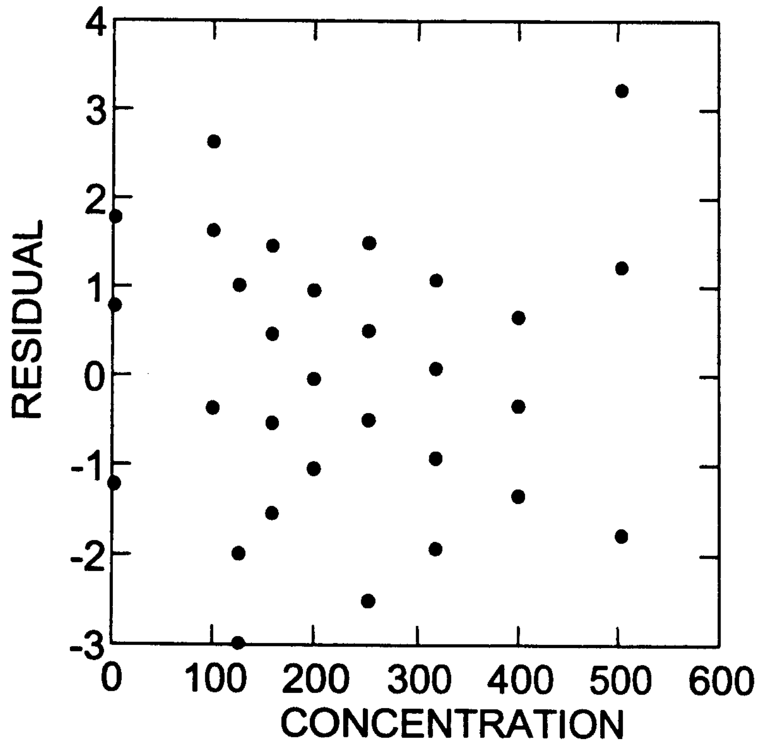
	G	X	B
G	1.000		
X	-0.785	1.000	
B	-0.622	0.942	1.000

Residuals have been saved.

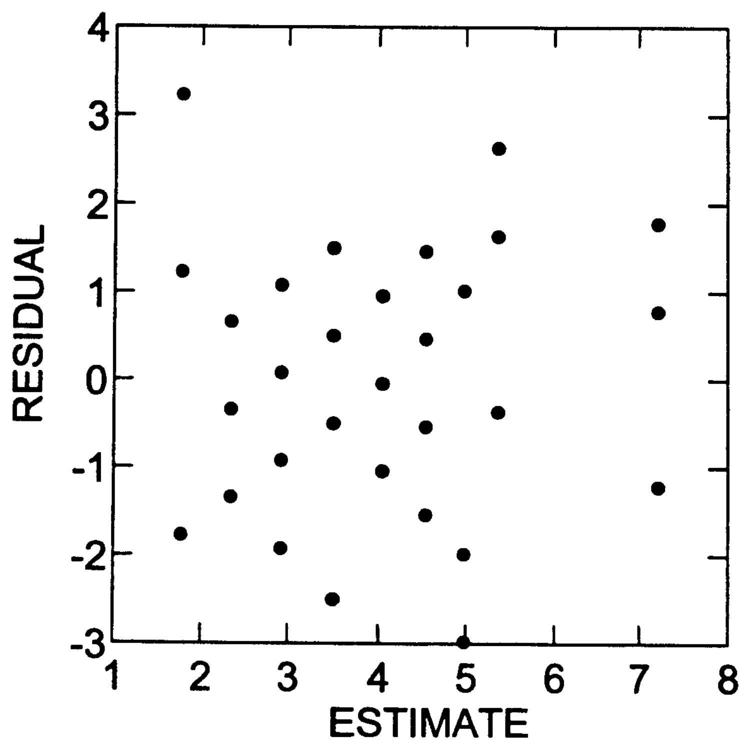
Effect of antimony on *F. candida* adult survival in aged/weathered SSL soil



Residuals



RESIDUAL
CONCENTRATION
4
3
2
1
0
-1
-2
-3
0
100
200
300
400
500
600



RESIDUAL
ESTIMATE
4
3
2
1
0
-1
-2
-3
1
2
3
4
5
6
7
8

ANOVA for Sb effect on *F. candida* adult survival in SSL soil.

```
SYSTAT Rectangular file
C:\DOCUME~1\RGKUPERM\MYDOCU~1\SYSTAT\ROMAN3\\NONLINRE\\NAVY\FOLSOMIA\COSBSDA2.SYD,
contains variables:
 CONCENTR      JUVENILES     ADULTS

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (9 levels)

2.5, 100, 126, 159, 200, 252, 318, 400, 504

Dep Var: ADULTS   N: 45   Multiple R: 0.772   Squared multiple R: 0.595


                                  -1
Estimates of effects  B = (X'X)    X'Y


                                   ADULTS

   CONSTANT                         4.089
   CONCENTR     2.5                 2.911
   CONCENTR     100                 2.511
   CONCENTR     126                 0.511
   CONCENTR     159                 0.111
   CONCENTR     200                -0.089
   CONCENTR     252                -0.889
   CONCENTR     318                -1.689
   CONCENTR     400                -1.889


                           Analysis of Variance

Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

CONCENTRATION             122.444    8       15.306       6.623     0.000

Error                      83.200   36        2.311

-------------------------------------------------------------------------------

Durbin-Watson D Statistic      2.250
First Order Autocorrelation   -0.132
COL/
ROW CONCENTRATION
  1  2.5
  2  100
  3  126
  4  159
  5  200
  6  252
  7  318
  8  400
  9  504
Using least squares means.
Post Hoc test of ADULTS
-------------------------------------------------------------------------------

Using model MSE of 2.311 with 36 df.
Matrix of pairwise mean differences:
```

	1	2	3	4	5
1	0.0				
2	-0.400	0.0			
3	-2.400	-2.000	0.0		
4	-2.800	-2.400	-0.400	0.0	
5	-3.000	-2.600	-0.600	-0.200	0.0
6	-3.800	-3.400	-1.400	-1.000	-0.800
7	-4.600	-4.200	-2.200	-1.800	-1.600
8	-4.800	-4.400	-2.400	-2.000	-1.800
9	-4.400	-4.000	-2.000	-1.600	-1.400
	6	7	8	9	
6	0.0				
7	-0.800	0.0			
8	-1.000	-0.200	0.0		
9	-0.600	0.200	0.400	0.0	

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.680	1.000			
3	0.017	0.045	1.000		
4	0.006	0.017	0.680	1.000	
5	0.004	0.010	0.537	0.836	1.000
6	0.000	0.001	0.154	0.305	0.411
7	0.000	0.000	0.028	0.069	0.105
8	0.000	0.000	0.017	0.045	0.069
9	0.000	0.000	0.045	0.105	0.154
	6	7	8	9	
6	1.000				
7	0.411	1.000			
8	0.305	0.836	1.000		
9	0.537	0.836	0.680	1.000	

ANOVA for Sb effect on *F. candida* juvenile production in SSL soil.

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTRATION (9 levels): 2.5, 100, 126, 159, 200, 252, 318, 400, 504

Dep Var: JUVENILES   N: 45   Multiple R: 0.909   Squared multiple R: 0.827

Estimates of effects $B = (X'X)^{-1} X'Y$

		JUVENILES
CONSTANT		88.444
CONCENTR	2.5	119.156
CONCENTR	100	74.956
CONCENTR	126	19.156
CONCENTR	159	30.356
CONCENTR	200	10.956
CONCENTR	252	-43.044
CONCENTR	318	-46.444
CONCENTR	400	-77.644

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTRATION	194549.511	8	24318.689	21.444	0.000
Error	40825.600	36	1134.044		

Durbin-Watson D Statistic 2.527
First Order Autocorrelation -0.264

COL/ ROW	CONCENTRATION
1	2.5
2	100
3	126
4	159
5	200
6	252
7	318
8	400
9	504

Using least squares means.
Post Hoc test of JUVENILES

Using model MSE of 1134.044 with 36 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	-44.200	0.0			
3	-100.000	-55.800	0.0		
4	-88.800	-44.600	11.200	0.0	
5	-108.200	-64.000	-8.200	-19.400	0.0
6	-162.200	-118.000	-62.200	-73.400	-54.000
7	-165.600	-121.400	-65.600	-76.800	-57.400
8	-196.800	-152.600	-96.800	-108.000	-88.600
9	-206.600	-162.400	-106.600	-117.800	-98.400

	6	7	8	9
6	0.0			
7	-3.400	0.0		
8	-34.600	-31.200	0.0	
9	-44.400	-41.000	-9.800	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.045	1.000			
3	0.000	0.013	1.000		
4	0.000	0.043	0.602	1.000	
5	0.000	0.005	0.702	0.368	1.000
6	0.000	0.000	0.006	0.001	0.016
7	0.000	0.000	0.004	0.001	0.011
8	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000

	6	7	8	9
6	1.000			
7	0.874	1.000		
8	0.113	0.152	1.000	
9	0.044	0.062	0.648	1.000
